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WITH INDEX FOR ALL THE VOLUMES

PREFACE

Since the revision of this work was first undertaken in 1907, a considerable amount of literature has appeared and many new methods of analysis have been devised. It, therefore, became desirable to issue a supplementary volume bringing the text, especially that of the earlier volumes, up to date. The new articles have, as far as possible, been written by the contributors to the earlier volumes, but, in a few cases, pressure of other work caused by the outbreak of war has made it necessary to entrust the revision to other hands.

A complete general index has also been prepared which is hoped will make the subject matter of the whole work more easily accessible.

The editors and publishers again wish to thank the various contributors for their valuable and willing coöperation in bringing the work to completion.

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APPENDIX.

ALCOHOLS.

By G. C. JONES, F. I. C., A. C. G. I.

METHYL ALCOHOL.

Detection and Estimation of Methyl Alcohol in Presence of Ethyl Alcohol.
—Since Vol. I appeared, many new methods have been described for this
purpose, but, so far as the writer is aware, only one of them marks a real
advance. By this method, due originally to Denigès,[1] the presence of 0.2%
of methyl alcohol (or about 4% of industrial spirit) in ethyl alcohol can be
detected with certainty within 20 minutes, and only twice as much time is
required to estimate the proportion of methyl alcohol with sufficient exact-
ness for most purposes. Denigès claims that the method will detect as little
as 0.1% of methyl alcohol in ethyl alcohol, and Simmonds[2] has shown that it
can be made even more sensitive. The writer's own experience confirms this,
but it will be seen from a subsequent paragraph that there is risk in attempt-
ing to push the sensitiveness too far, and the method as now to be described
will not detect much less than 0.2% of methyl alcohol expressed as a percent-
age of the total alcohols. This is sensitive enough for almost any purpose,
as the addition of less than 5% of industrial spirit to ethyl alcohol would
probably represent too small a saving to the sophisticator to be worth his
while. The subsequent notes show the direction in which the test should
be modified to increase its sensitiveness, should this be necessary.

Simmonds (*loc. cit.*) describes the test as follows: "The alcoholic liquid
is first purified, where necessary, either by the method of Thorpe and Holmes
(Vol. I, p. 129) or by other suitable means. It is then diluted with water
until it contains 10% of total alcohol by volume.

"To 5 c.c. of this prepared liquid, contained in a wide test-tube, are
added 2.5 c.c. of permanganate solution (2%), and then 0.2 c.c. of strong
sulphuric acid. When the reaction has proceeded for 3 minutes, 0.5 c.c. of
oxalic solution (9.6 grm. crystals in 100 c.c.) is added. On shaking, the
liquid becomes clear and nearly colourless. 1 c.c. of strong sulphuric
acid is now run in and well mixed with the solution, which is finally treated
with 5 c.c. of Schiff's reagent. A violet colour is developed in the course
of a few minutes unless mere traces of methyl alcohol were present, when 20
or 30 minutes may be required.

[1] *Compt. rend.*, 1910, 150, 832.
[2] *Analyst*, 1912, 37, 16.

"A preliminary experiment carried out as described serves to detect the presence of methyl alcohol and to give some idea of the quantity. According to the indications thus obtained, another part of the prepared liquid is further diluted, if necessary, with ethyl alcohol of 10% strength until it contains from 0.001 to 0.004 grm. methyl alcohol in 5 c.c., and the experiment is repeated side by side with two or more standards for comparison. These contain 0.001, 0.002, 0.003, etc., grm. methyl alcohol in 5 c.c. of 10% ethyl alcohol."

The above description of the test requires so little amplification that the writer has preferred to leave it in the words of Simmonds, who first sought to establish the test here.   Simmonds does not give the formula of the Schiff's solution to be used, nor does he, in the writer's view, lay sufficient stress on the necessity of measuring all the reagents used, a point of great importance.   Curiously enough, the strength of the oxalic acid, which is of least importance, he states with great precision.   He points out, it is true, that the function of the sulphuric acid added immediately before the Schiff's reagent is to suppress any colouration due to acetaldehyde, but does not say that the intensity of colouration due to a stated amount of formaldehyde is closely dependent on the final concentration of acid, as it actually is.   Again, the amount of formaldehyde formed from methyl alcohol depends on the exact conditions of oxidation, and even ethyl alcohol itself may yield formaldehyde if the conditions are not strictly controlled.   This last fact has settled the fate of many similar methods.   In first describing this test Denigès pointed out that the use of too high a concentration of sulphuric acid with the permanganate would give rise to formaldehyde, even with pure ethyl alcohol.

The formula adopted in the preparation of the Schiff's solution is important, as it affects the final concentration of acid.   The first (strongly acid) solution described on page 197 of Vol. I is quite useless for the purpose, as are many less acid ones, e.g., that of Mohler,[1] perhaps that most used in the examination of potable spirits.   The other solution described in Vol. I, made from magenta base and sulphurous acid, serves well and was used in all the experiments described below.

A 9.6% solution of oxalic acid, as recommended by Simmonds, deposits crystals at ordinary temperatures, but with a cold saturated solution of oxalic acid, Schiff's solution of the character recommended above, and precise measurement of quantities, Simmonds' directions may be followed.

How nearly the quantities need to be measured and how they should be varied to increase or diminish the sensitiveness of the test will be evident from the following results obtained in the writer's laboratory.

The amount of permanganate used must be rigidly adhered to in quantitative work, since it determines the amount of formaldehyde formed and the final intensity of colour.   The use of 2 c.c. in place of 2.5 c.c. reduces the final

[1] Frémy's *Encyclopédie Chimique*, 1892, X, 11, 278.

colour about 30%, whilst the use of 5 c.c. more than doubles the sensitiveness of the test, when all other conditions are kept the same, except that the use of 5 c.c. of permanganate necessitates the subsequent use of about 1 c.c. instead of 0.5 c.c. of oxalic acid solution. Provided all the other standard conditions are rigidly adhered to, this is the simplest and safest way of increasing the sensitiveness of the test, as ethyl alcohol under these conditions yields no formaldehyde and no colour, but the permissible latitude in all the other measurements necessarily becomes narrower.

The amount of sulphuric acid added with the permanganate is less important. The amount of formaldehyde produced is greater with more acid, but, within the limits 0.1–0.3 c.c., the final results are indistinguishable, owing to the compensating effect of the higher final concentration of acid reducing the intensity of colour due to a definite quantity of formaldehyde. Quantities in excess of 0.5 c.c., however, might lead to formaldehyde being produced from ethyl alcohol itself.

The time allowed for oxidation, provided it be not less than 3 minutes, appears to be without effect. The influence of temperature may not be negligible, but the point was not investigated, as this would clearly be constant in any one set of experiments.

Not much less than 0.5 c.c. of a cold saturated solution of oxalic acid will reduce the excess of permanganate in the cold, faintly acid solution, but larger quantities, up to 1 c.c., appear to have no appreciable influence on the results.

The subsequent addition of sulphuric acid must be as nearly as possible the same in any one set of experiments and is most conveniently made 1 c.c. as directed by Simmonds. With only 0.6 c.c. added, the acetaldehyde derived from pure ethyl alcohol will give a distinct colouration with Schiff's solution of the character described. With 0.75 c.c. or more, pure ethyl alcohol gives no purple or even blue colour, provided all the other standard conditions are rigidly adhered to, but it is unwise to reduce the amount below 1 c.c. except in very special circumstances, when it is necessary to make the test as sensitive as possible. In such cases, the sensitiveness of the test can be increased about 40% by using only 0.75 c.c. of acid, but very careful control experiments then become necessary. On the other hand, not more than 1 c.c. of acid should be used, as the use of so much as 1.25 c.c. reduces the sensitiveness of the test by about 30%, whilst 1.5 c.c. reduces it 50% and 2 c.c. nearly 90%.

Finally, the amount of Schiff's solution taken is not without influence. If 10 c.c. be taken instead of 5 c.c., the concentration of acid is so much reduced that even the acetaldehyde from ethyl alcohol develops a colour. On the other hand, the use of only 2 c.c. in place of 5 c.c. reduces by about 90% the amount of colour developed by a fixed quantity of formaldehyde.[1]

[1] *Analyst*, 1915, 40, 218.

ETHYL ALCOHOL.

Estimation of Alcohol by the Ebullioscopic Method.—This method
(Vol. I, p. 126) has been considerably developed of late, especially in
France, where it is used for official purposes. The latest form of apparatus,
a proprietary design, is costly, but it gives results of great exactness, and
is operated simply; consequently it may be expected to find increasing use
in laboratories where large numbers of alcohol estimations must be made.
It has been described by J. C. Cain.[1]

ERRATA, VOL. I.

Page 14. In the tables of sp. gr. corresponding with degrees Baumé given by the
United States Dept. of Agriculture Bull., No. 107 (Revised, 1912), pp. 221-224, which are
based on the work of Mategczek and Scheibler (*Zeit. Ver. Deuts. Zuckerind.*, 1865, **15**,
580; 1874, **24**, 827), the degrees Baumé are on the "old" Rational Scale of Kolb, and
refer to 17.5° C. On page 292 of Vol. I the values given are Baumé degrees on the Gerlach
scale at 17.5°, sp. gr. $= \dfrac{146.8}{146.8 - n}$ at 17.5°.

Page 15, line 15 from bottom, "in the other cases" should read "in other cases."

Page 17, line 15 from bottom, "is destroyed" should read "disappears."

Page 20, line 13 "577.9" should read "757.9."

Page 26, line 10 from bottom "Fig. 12" should be "Fig. 13."

Page 29, lines 4, 9 and 11 from bottom, for "C" read "L"; line 7 from bottom, for
"tube D" read "vessel B."

Page 33, line 9, for "Fig. 12" read "Fig. 13."

Page 39, line 8, for "Vol. II" read "Vol III."

Page 44, line 7 from bottom; for "is" read "in."

Page 46, line 3, for "in" read "on."

Page 48, line 8 from bottom, for "quire" read "quite."

Page 55, line 17 from bottom, for "Vol. II" read "Vol. III."

Page 56 line 5 from bottom, for "comyany" read "Company."

Page 59, line 11 from bottom, for "is not well adapted to" read "unsuitable for."

Page 86, line 12, for "met, hyl" read "methyl."

Page 92, line 11, for "Ester," read "Esters."

Page 94, line 1, for H_2CrO_4 read CrO_3.

Page 96, line 13, for semicolon put comma and line 14, after reached, insert semicolon.

Page 98, line 16, insert comma after "respectively"; line 23, after carbonate insert "are"
line 10 from bottom, for "rodium" read "sodium."

Page 105, lines 17 and 18 from bottom should be transposed.

Page 108, lines 16 and 17 from bottom should be transposed.

Page 109, lines 8 and 9 should be transposed.

Page 110, line 15 from bottom, for "which alcohol has" read "whose alcohol had."

Page 129, line 2 from bottom, for "one" read "a single."

Page 129, bottom line, for "extraction spirit" read "extraction with petroleum spirit."

[1] *Chem. News*, 1914, **109**, 37.

MALT AND BREWING MATERIALS.

BY G. C. JONES, F. I. C., A. C. G. I.

MALT.

Extract.—On page 134 of Vol. I, directions are given to grind malt in a "Seck" mill set at 25° on the arbitrary scale attached to that instrument. It has been found that Seck mills, although sold as standard instruments at a very high price, differ widely amongst themselves. Since, however, large numbers have been installed in brewing laboratories, it has been decided by the Malt Analysis Committee of the Institute of Brewing[1] to continue their use for the present but, instead of relying on the arbitrary scale, to set each mill so that the rollers are 0.5 mm. apart, as determined by a feeler gauge.

Diastatic Power.—The most frequent cause of divergence between the results of different workers is the use of starch solutions of different degrees of acidity. There are other, less easily avoidable sources of error (cf. *J. Inst. Brewing*, 1908, 14, 12), but this one is readily avoided. The 2% starch solution should on no account be alkaline when tested hot with alizarin, nor should it be so strongly acid that 200 c.c. require more than 0.2 c.c. of deci-normal caustic alkali to neutralise it. Two drops of a suspension (1 : 200) of alizarin paste confer a scarcely perceptible yellow tint on the acid or "neutral" solution, but the change to incipient purple on adding the least excess of alkali is quite sharp.

The temperature at which the 5% malt extract is made may be 70± 5° F. without influence on the results, but the temperature of the starch digestion must be precisely 70° and the time precisely 1 hour. An error here of 1° or 2' introduces an error of about 3% in the results.

Ferrous thiocyanate is not obtainable commercially and, if it were, would almost certainly be contaminated with ferric salts. The indicator referred to in Vol. I is made as follows:

One grm. of ferrous ammonium sulphate is dissolved in 10 c.c. of cold water, 1.5 grm. of ammonium thiocyanate is added and, when this has dissolved, 2.5 c.c. of concentrated hydrochloric acid. With the purest available materials, the solution, even when freshly prepared, will usually have a pink tint. This is destroyed by a trace of zinc dust and the solution is filtered. After being kept some time, it re-acquires a red colour, which is again discharged by the use of zinc dust. In this way the indicator may be used for

[1] *J. Inst. Brewing*, 1910, 16, 531.

several days, but finally becomes too insensitive and must be replaced by a freshly prepared solution.

Roasted Malt and Barley.

A uniform practice in analysing these products has now been agreed on[1] which differs considerably from the methods described in Vol. I (p. 142).

Extract.—A little over 50 grm. is finely ground in a coffee mill, and exactly 50 grm. of the ground product are mixed with about 350 c.c. of boiling distilled water and the mixture kept in a boiling water bath for an hour. It is then cooled, made up to 515 c.c., filtered and its sp. gr. at 60°/60° taken. The excess gravity (over water = 1,000) multiplied by 3.36 gives the extract in brewers' lb. per standard quarter of 336 lb. Black barleys and malts are not commonly purchased on the basis of the standard quarter of 336 lb., various weights and measures being employed, but the Malt Analysis Committee of the Institute of Brewing recommends the above uniform method of stating analytical results, leaving subsequent calculation to whom it may concern.

Colour.—20 c.c. of the above extract, which, if not brilliant, must be refiltered, are diluted to 1,000 c.c. and the colour read in a 1-in. cell, using Lovibond's tintometer and glasses of "Series 52."

Brown and Crystal Malts.

No directions for the analysis of these materials were given in Vol. I. The following uniform methods have now been agreed on.[2]

, **Extract.**—50 grm. are ground in a Seck mill, so set that there is a distance of 0.5 mm. between the rollers, as determined by a feeler gauge. The grist is mashed with 300 c.c. of distilled water at 158° F. and 100 c.c. of cold-water malt extract[3] previously heated to 150° F. The mixture is kept for an hour at 150° F., then cooled to 60° F., made up to 515 c.c., filtered and the sp. gr. of the filtrate taken at 60°/60° F. Simultaneously, 100 c.c. of the cold-water malt extract, mixed with 300 c.c. water, are digested for an hour at 150° F., then cooled to 60° F., made up to 500 c.c., filtered and the sp. gr. of the filtrate taken. The difference between the sp. gr. (water = 1,000) of this filtrate and that obtained in the experiment with brown or crystal malt, multiplied by 3.36, gives the extract of the malt in brewers' lb. per standard quarter of 336 lb. Brown and crystal malts are not commonly purchased on the basis of the standard quarter, various weights and measures being employed, but the above uniform method of stating results is recommended.

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[1] *J. Inst. Brewing*, 1910, **16**, 532.
[2] *J. Inst. Brewing*, 1910, **16**, 532.
[3] The cold-water malt extract is made by digesting malt of diastatic power 30 to 40° Lintner with three times its weight of distilled water for 1 hour at 60°–70° F., and then filtering.

Colour.—20 c.c. of the above extract, which must be brilliant, are diluted to 100 c.c. and the colour read in a 1-in. cell, using a Lovibond tintometer and glasses of "Series 52."

Caramel.

Extract.—10 grm. are dissolved in distilled water, made up to 100 c.c., filtered and the sp. gr. of the filtrate determined at 60°/60° F. The excess gravity (water = 1000) multiplied by 2.24 gives the extract in brewers' lb. per 2 cwt.

Colour.—10 c.c. of the above solution are diluted to 1,000 c.c., and the colour read in a 1-in. cell, using a Lovibond tintometer and glasses of "Series 52."

Ash.—3 grm. are treated with 2 c.c. of sulphuric acid in a tared dish, which is then heated over a flame until intumescence is complete, when it is transferred to a muffle and the contents are incinerated at a low red heat. It is usual to deduct 10% from the weight of the ash found, as in the analysis of sugars, though the mineral constituents of caramel are so frequently mainly sulphates that no correction, or at most a very small one, is really needed.

Iron is nearly always present in detectable amount and, since brewers object to more than a minute trace of iron in their materials, it is usual to estimate the iron in the ash by dissolving the latter in hydrochloric acid, adding much thiocyanate and comparing the colour produced with standards containing known amounts of ferric iron and the same concentration of acid and thiocyanate.

Caramel for use in brewing should throw down no sediment when mixed with beer. The test is usually continued for 24 hours. If prolonged beyond this period, as is sometimes worth while, some beer without caramel should be set up alongside, lest a sediment due to the beer itself be attributed to the caramel.

Invert Sugar.

Invert sugar, made by hydrolysing raw cane sugars with acid, is an important brewing material and is subjected in brewing laboratories to a much more exhaustive scheme of analysis than is to be found under this heading in the section on Sugars.

Invert sugar made from refined sugar lacks the lusciousness and other characteristics desirable in a brewing sugar, so that raw cane sugars are generally used. In addition to invert sugar, uninverted saccharose and water, therefore, commercial invert contains from 0.2 to 0.7% of albuminoids, from 3 to 6% of unfermentable organic matter and from 1 to 3.5% of mineral matter, the latter being partly derived from the raw material and partly introduced as calcium carbonate to neutralise the acid used in effecting

hydrolysis. Sulphuric acid is generally employed as hydrolyst because the comparative insolubility of calcium sulphate makes it possible to eliminate most of the mineral matter introduced for the purpose of neutralisation.

Raw beet sugar could not be used for the production of brewers' invert, on account of the objectionable flavour of the secondary constituents. No such objection would attach to the use of hightly refined beet sugar, but highly refined sugars are not used for the reasons already stated. Occasionally invert sugar is made from a mixture of raw cane sugar and high-grade raw beet sugars (first runnings) and the origin of such invert sugar is not readily detected by the palate or nose. It is, however, desirable to exclude it from the brewery, and this can usually be done by limiting the permissible percentage of albuminoids, which is higher in beet than in cane products. Brewers' invert is supplied in three grades, and it is reasonable to require them to contain less than the following percentages of albuminoids: No. I, 0.3%; No. II, 0.5%; and No. III, 0.75%. A good No. III will comply with the standard here set up for No. I, so that the above limits cannot be unduly stringent.[1]

The analysis of commercial invert includes the following determinations: dextrose, lævulose, saccharose, albuminoids, ash and water. The difference between the sum of these and 100 is returned as "other organic matter." The brewers' extract per 2 cwt. is also an important figure, always determined, and the colour may be.

Ash.—This is determined as in caramel (q.v.) and it is customary to deduct 10% from the weight of the sulphated ash as with other sugars, although the correction is no doubt too large for a product inverted with sulphuric acid.

Water and Brewers' Extract.—25 grm. are dissolved in distilled water, the mixture made up to 250 c.c., filtered, and its sp. gr. determined at 60°/60°. The excess gravity (water = 1,000) multiplied by 2.24 gives the extract in brewers' lb. per 2 cwt.

Water.—A 10% solution of pure invert sugar has a sp. gr. of 1,038.7 and, in solutions of approximately this concentration, the excess sp. gr. is very nearly proportional to the concentration. The composition of a solution of pure invert sugar can therefore be calculated by dividing the excess gravity by the "solution factor" 3.87. The solution factor of uninverted sucrose is 3.86, and that of the other organic solids of commercial invert probably not very different, whilst their amount is small. The solution factor of the ash constituents, on the other hand, approximates to 8 and in brewery laboratories is usually assumed to be double that of the organic solids. The percentage of total solids in the invert is therefore arrived at by dividing the excess gravity of the 10% solution by 0.387 and subtracting the percentage of ash. The difference between the result so found and 100 is the percentage of water in the sample.

[1] cf. Ling, *J. Inst. Brewing*, 1914, **20**, 185.

Albuminoids.—Nitrogen is determined by Kjeldahl's method and the result multiplied by 6.3. If unduly high, a separate determination of nitrate nitrogen should be made before condemning the sample on its high content of "albuminoids."

Invert Sugar and Saccharose.—These can be estimated by a method, worked out by Morris[1] who first devised a means of overcoming the interference of optically active and reducing non-sugars. The following method, based on that of Morris, is easier of execution and has been found by Ling (its originator) and the writer to be capable of a somewhat higher degree of accuracy. It depends on the volumetric estimation of the reducing sugars and the use of the table constructed by Ling and the writer.[2] The table was constructed from experiments made with Fehling's solution of which 10 c.c. required 25.65 c.c. of 0.2% pure invert sugar. If another worker finds that 10 c.c. of his Fehling's solution requires under his conditions only 25 c.c. of 0.2% invert sugar, he will need to reduce the numbers in columns D, L, I and M and increase those in columns D', L', I' and M' proportionately. The maltose columns are for use in the analysis of commercial glucose (*vide supra*).

To make clear the calculations involved, the following description of the method is accompanied by a worked example.

The rotation of the 10% solution, prepared for the estimation of water and brewers' extract, is observed in a 200 mm. tube in a Ventzke-Scheibler half-shadow polarimeter. 10 c.c. of this solution are diluted to 500 c.c. and the reducing power of this 0.2% solution determined by titration with 10-c.c. portions of Fehling's solution, using ferrous thiocyanate as indicator (cf. Vol. I, p. 136, for method, and this Vol., p. 5, for preparation of indicator).

In a particular case, a commercial invert sugar gave a reading in 10% solution in a 200-mm. tube of −4.7 divisions, and 10 c.c. of Fehling's solution required 36.45 c.c. of 0.2% solution for reduction.

10 c.c. of the 10% solution are diluted to about 150 c.c. and boiled for 1 minute with 30 c.c. of $N/2$ hydrochloric acid to invert the small amount of saccharose always present in commercial invert sugar. The mixture is cooled, neutralised with 30 c.c. of $N/2$ sodium hydroxide, diluted to 500 c.c. and titrated against Fehling's solution.

In the case cited, 10 c.c. of Fehling's solution required 35.95 c.c. of the completely inverted 0.2% solution. From this result and the reducing power of the 0.2% solution before inversion, the percentage of Saccharose is calculated. Reference to column 1 of the table shows that the reducing power of the inverted solution corresponds to the presence of 0.1460% of apparent invert sugar, that of the uninverted solution to 0.1441%. The difference, 0.0019, is the measure, in terms of invert sugar, of the saccharose

[1] *J. Inst. Brewing*, 1898, 4, 162.
[2] *Analyst*, 1908, 33, 160. (See under Sugars, page 39.)

in 0.2 grm. of the sample, which therefore contained (0.0019 $\times$ 0.95 $\times$ 500 =) 0.9% saccharose.

25 grm. of the original sample are dissolved in about 200 c.c. water, to which about 5 c.c. of yeast decoction and 3 grm. of washed, pressed yeast are added, and the mixture is allowed to ferment at about 70° C. for 3–4 days. A little alumina cream is then added, the mixture made up to 250 c.c., filtered and its rotation and reducing power determined.

In the case cited, the fermented 10% solution gave a reading in a 200 mm. tube of −0.1 division, and 40 c.c. was required to reduce 10 c.c. of Fehling's solution. Opposite 40 c.c. in column I of the table is the number 0.1319, the reducing power in terms of invert of the unfermentable matter in 10% solution. In 0.2% solution, therefore, the unfermentable matter would raise the apparent content of invert sugar 0.0026%. The table also shows that in a concentration such that 10 c.c. Fehling's solution require 36–37 c.c., a difference of 0.0037% of invert sugar makes a difference of 1 c.c. in the burette reading. Unfermentable reducing substances equivalent to 0.0026% invert would therefore reduce it 0.70 c.c.   10 c.c. of Fehling's solution would therefore require (36.45 + 0.70 =) 37.15 c.c. of the 0.2% solution, if this were free from unfermentable reducing substances, or 1 grm. of the sample contains dextrose and lævulose equivalent to 10 ÷ (37.15 $\times$ 0.002) = 10 ÷ 0.0743 = 134.6 c.c. Fehling's solution.

In concentrations such as that in which the first reduction experiment was made—namely where 10 c.c. Fehling's solution require 36.45 c.c. of sugar solution—1 grm. dextrose = 196.6 c.c. Fehling's solution and 1 grm. lævulose = 183.8 c.c. Fehling's solution. If the percentage of dextrose in the sample be represented by D and the percentage of lævulose by L, it follows that

$$1.966\,D + 1.838\,L = 134.6 \tag{1}$$

The sample was found to contain 0.9% saccharose. A 0.9% solution of saccharose gives a reading of (3.85 $\times$ 0.09 =) + 0.3 division when read in a 200-mm. tube in a Ventzke-Scheibler polarimeter. The actual reading (−4.7 must therefore be corrected for this amount, as well as for the reading of the unfermentable residue (−0.1), in order to arrive at the reading due to dextrose and lævulose alone, −4.7 − 0.3 − (−0.1) = − 4.9 divisions. Since 1% solutions of dextrose or lævulose give readings of 3.05 and −5.32 divisions respectively, it follows that

$$0.305\,D − 0.532\,L = − 4.9 \tag{2}$$

From equations (1) and (2), D = 39.0 and L = 31.5%.

Starch Sugars.

Glucose chips contain in addition to dextrose, maltose (nil to 12%), dextrin (up to 14%), water (usually 13–14%), small amounts of mineral

matter (0.5–2%) and still smaller amounts of albuminoids (0.2–0.6%). Glucose syrup or dextrin-maltose usually contains rather less than 25% of dextrose, rather more maltose,[1] over 30% of dextrin, about 18% of water and traces of mineral matter and albuminoids.

As regards ash, albuminoids, water and brewers' extract, these sugars are analysed like commercial invert sugar (*q.v.*).

Dextrose and maltose are estimated in a manner similar to that employed for the estimation of dextrose and lævulose in invert sugar, except that no inversion experiment is necessary and that the equations to be used are:

$$aD + 1.225M = F, \tag{1}$$
$$0.305D + 0.798M = R, \tag{2}$$

where a is a coefficient found by reference to the table on page 39 after experiment, as in the analysis of invert sugar, F is the number of c.c. of Fehling's solution corresponding to 1 grm. of the starch sugar, duly corrected for the reducing power of the unfermentable residue, and R is the rotation in divisions Ventzke (200 mm. tube) of a 10% solution of the sugar, duly corrected for the rotatory power of the unfermentable residue. The coefficient of M in equation (1) is a constant, independent of the concentration.

The difference between 100 and the sum of the percentages of dextrose, maltose, water, ash and albuminoids may be, and usually is, returned as "dextrinous carbohydrates and other organic matter." The percentage of actual dextrin is approximately given by dividing the rotation (Ventzke, 200 mm. tube) of the fermented 10% solution by 1.166.

Beer.

Determination of Original Gravity.—The Finance Act of 1914 (Session 2) substitutes for the table, reproduced on page 153 of Vol. I, a new table, of which a copy is given below.

The figures in the new table, which is based on experiments made *ad hoc* by Sir Edward Thorpe and Dr. Horace Brown, are for the most part higher—and, over an important part of the table, nearly 2° higher—than those in the old one, which has long been known to give low results (cf. Vol. I, 155). On the average, the new table will allow original gravities to be determined accurately, but in some cases it will overestimate and in others underestimate original gravities. This is inevitable, as the true relation between spirit indication and degrees of gravity lost varies with the composition of the wort and with the individual brewery. To meet cases where it may overestimate, the Finance Act directs that 0.75° be deducted in all cases from the original gravity as deduced from the table. This, of course,

[1] Kluyver (*Biochemische Suikerbepalingen*, Leiden, 1914) states that a series of analyses, made by discriminating yeasts, of commercial glucose syrups showed the presence of 15 to 20% of maltose in the products he examined.   W. A .D.

applies to work connected with the departments of Customs and Excise. For purposes of brewery control, it may be found that a smaller correction, or one of contrary sign, or none, is needed.

SPIRIT INDICATION TABLE SHOWING DEGREES OF GRAVITY LOST IN MALT WORT DURING FERMENTATION.

Degrees of spirit indication	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0	0.00	0.42	0.85	1.27	1.70	2.12	2.55	2.97	3.40	3.82
1	4.25	4.67	5.10	5.52	5.95	6.37	6.80	7.22	7.65	8.07
2	8.50	8.94	9.38	9.82	10.26	10.70	11.14	11.58	12.02	12.46
3	12.90	13.34	13.78	14.22	14.66	15.10	15.54	15.98	16.42	16.86
4	17.30	17.75	18.21	18.66	19.12	19.57	20.03	20.48	20.94	21.39
5	21.85	22.30	22.76	23.21	23.67	24.12	24.58	25.03	25.49	25.94
6	26.40	26.86	27.32	27.78	28.24	28.70	29.16	29.62	30.08	30.54
7	31.00	31.46	31.93	32.39	32.86	33.32	33.79	34.25	34.72	35.18
8	35.65	36.11	36.58	37.04	37.51	37.97	38.44	38.90	39.37	39.83
9	40.30	40.77	41.24	41.71	42.18	42.65	43.12	43.59	44.06	44.53
10	45.00	45.48	45.97	46.45	46.94	47.42	47.91	48.39	48.88	49.36
11	49.85	50.35	50.85	51.35	51.85	52.35	52.85	53.35	53.85	54.35
12	54.85	55.36	55.87	56.38	56.89	57.40	57.91	58.42	58.93	59.44
13	59.95	60.46	60.97	61.48	61.99	62.51	63.01	63.52	64.03	64.54
14	65.10	65.62	66.14	66.66	67.18	67.70	68.22	68.74	69.26	69.78
15	70.30	70.83	71.36	71.89	72.42	72.95	73.48	74.01	74.54	75.07
16	75.60	....	....	....	....	....	....	....	....	....

ERRATA IN VOL. I.

Page 161, line 15, for "hopsarrogates," read "hop surrogates," 164, line 13, for "saccharine," read "saccharin."

WINES AND SPIRITS.

By G. C. JONES, F. I. C., A. C. G. I.

Physico-chemical Analysis of Wines.—The time has not yet come for the description, in a general work like this, of the physico-chemical methods of P. Dutoit and M. Duboux. Nothing less than a detailed description, occupying much space, could serve any useful purpose, and this would scarcely be justified, seeing that the methods are not yet used in this country, so far as the writer is aware, whilst they are ignored by all German writers, e.g., by the author of the section on Wines in the most recent edition of Lunge's *Technical Methods of Analysis*.

However, the application by Dutoit and Duboux of their methods to the investigation of wine dates back at least to 1908 and is of general interest, if only for its originality. It is quite probable that the methods may come into general use in laboratories where a large amount of routine work has to be conducted. Such work on wine is not often undertaken in Great Britain, but those who refer to this book have a right to expect at least a reference to work which may become important before another edition appears. Fortunately, the authors have recently published a small book,[1] which makes it unnecessary to give references to their original papers, some of which appeared in Swiss periodicals, not readily accessible.

Briefly, their methods consist in titrating the wine with appropriate reagents and taking successive conductivity measurements, the results being plotted on a system of rectangular coordinates. In this way, curves are obtained which show distinctly the saturation points. Not only can chlorides, sulphates, phosphates, lime, total alkalinity, total acidity, ash and ammonia be thus estimated, but also tartaric, malic and succinic acids.

The book referred to embodies their work up to 1912, since when only one paper has been published.

Estimation of Tartaric, Malic, Succinic and Other Organic Acids in Wine.—The estimation of tartaric acid and tartrates so seldom figures in an English report on a wine that a mere reference to the tedious methods of Halenke and Moslinger was given in Vol. I. For the same reason, no extended reference can be given here to improved methods for the estimation of tartaric, malic and other acids in musts, wines and the like. But the last 5 years have witnessed several more or less successful attempts to solve the difficult problem of estimating these acids when present together; for these methods, see under Tartaric Acid, pages 104–109.

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[1] *L'Analyse des Vins par Volumétrie Physico-Chimique.* Lausanne, Rouge et Cie.

ERRATA IN VOL. I.

Page 165, line 2, for A. C. S. I. read "A. C. G. I."
Page 165, line 21, from bottom, for "difference," read "differences."
Page 168, line 13, after "wines" insert "in which."
Page 174, line 18, insert "acid" after "sulphurous."
Page 175, line 20 from bottom, for Vol. III, read Vol. V.
Page 175, line 8 from bottom, for "forms," read "form."
Page 191, line 8 from bottom, for "bearing," read "leaving."
Page 196, line 8, for "in," read "under."
Page 205, line 2, for "Schlighting," read "Schlichting."

YEAST.

By EMIL SCHLICHTING and H. WINTHER.

General.—Yeast must be considered from two points of view, as a plant and as a ferment, based upon the fact that, while the yeast cells develop and propagate, the yeast has only little or no fermentative activity.

Physical Properties.—According to Schönfeld, Hinrichs and Rossmann,[1] the four main characteristics of a "*top fermenting yeast*" are: (1) branched budding fermentation; (2) little fermentation of melitriose; (3) formation of surface yeast at room temperature; (4) milky mixture in water.

Schönfeld and Hirt[2] point out that considerable difference exists among the various yeasts in their mode of settling or sedimentation, some separating out in heavy lumps while others subside as a fine, loose dust. According to these authors, lumpy yeasts contain a larger percentage of phosphoric acid and magnesia. The distinctive character of settling is also greatly influenced by the nature of the proteins contained in the nutritive medium. The more complex their molecular composition the greater the tendency of the yeast to separate in solid, lumpy formation.

Classification.—In the latest systematizing of yeasts, Hansen[3] distinguishes between the following groups:

Saccharomyces.
Zygosaccharomyces.
Saccharomycodes.
Saccharomycopsis
Pichia.
Willia.

The *Schizosaccharomyces* are not included in this family. Simultaneously with these changes in the grouping of yeasts, several of the known species have been given new names. The most important of these changes are:

Old name.	New name.
Saccharomyces Ellipsoideus I.	*Saccharomyces Ellipsoideus.*
Saccharomyces Ellipsoideus II.	*Saccharomyces Turbidans.*
Saccharomyces Pastorianus I.	*Saccharomyces Pastorianus.*
Saccharomyces Pastorianus II.	*Saccharomyces Intermedius.*
Saccharomyces Pastorianus III.	*Saccharomyces Validus.*
Saccharomyces Membranefaciens.	*Pichia Membranefaciens.*
Carlsberg Yeast I.	*Saccharomyces Carlsbergensis.*
Carlsberg Yeast II.	*Saccharomyces Monacensis.*

Chemical Composition.—The characteristic odour of yeast is ascribed[4] to a colourless ethereal oil, which, in concentrated form, resembles the flavour of hyacinth.

[1] *Wochenschrift Brau.*, 1910, 27, 493.
[2] *Wochenschrift Brau.*, 1912, 29, p. 174.
[3] F. Lafar, *Techn. Mykologie*, IV, p. 172.
[4] Delbrück, *Brau. Lexikon*, 1910, 455.

Henneberg has found that a definite relation exists between the amount of protein and glycogen present in yeast and that when the former rises above 53%, the latter is reduced to almost nil.

Salkowski[2] has determined the amount of yeast gum to be about 5%. He has further shown that the gum consists of a dextro-mannan, which on hydrolysis forms dextrose and mannose.

Fermenting Enzyme.—A. V. Lebedew[3] has demonstrated that the active fermenting enzyme can be extracted from dried yeast by simple maceration with water at a temperature from 25° to 30° C. for a period of 2 hours.

The process of decomposition of sugar into alcohol and carbon dioxide is a very complicated one.[4] According to Harden and Young, the transformation is carried out by the zymase proper and its co-enzyme, which is considered to be an easily saponified ester of phosphoric acid.

The destruction of the activity of pressed yeast juice is mainly ascribed to the action of a saponifying enzyme or lipase upon the co-enzyme; this is also accompanied by the action of a proteolytic enzyme, endotryptase, upon the zymase. Inactive yeast juice may be regenerated by the addition of yeast water if such addition is made shortly after the inactivity has set in.

Testing of Yeast.—C. Nagel[5] has modified the Hayduck method of estimating the fermenting power of yeast for baking purposes by changing the composition of the solution employed in the following manner:

400 c.c. of 10% cane-sugar solution to which are added 2 grm. of potassium hydrogen phosphate; 1 grm. of ammonium hydrogen phosphate; 0.25 grm. magnesium sulphate; 0.20 grm. calcium sulphate. The valuation of the yeast is based upon the amount of carbon dioxide generated within 2 hours, and may be expressed as follows:

	Fermenting power.
1000 c.c. of CO_2	good
800 to 1000 c.c. of CO_2	medium
less than 800 c.c. of CO_2	poor

Dry Yeast.—According to Hayduck and Bulle,[6] when in the drying process of the yeast proper, the moisture content drops below 25 or 30%, all vegetative cells are killed. That sometimes such yeast will start to grow again, is entirely due to some surviving spores.

In order to produce a dried yeast with about 90% of living cells, it should be mixed with 10% of cane sugar at a temperature of 50° and the mixture should be dried at this temperature on gauze, the period of drying being about 3 hours. The drying may be accelerated by a strong air current; as the sugar added is fermented during the drying process, little or no sugar will be found in the finished product.

[1] *Wochenschrift Brau.*, 1910, **27**, 429.
[2] *Chem. Centralbl.*, 1911.
[3] *Annales de l'Institut Pasteur*, 1912, **26**, 8.
[4] Delbrück, *Brauerei Lexikon*, 1910, 865.
[5] *Brennerei Zeitung*, 1911.
[6] *Wochenschrift Brau.*, 1912, vol. **29**, 489.

The drying of yeast has lately become a general practice especially in Europe for economic reasons and various forms of apparatus have been constructed for this purpose. It has been definitely established that yeast after being freed from its bitter substances is an admirable substitute for meat in the production of extracts, bouillon, etc. The nitrogenous substances in this *"nutrient yeast"* are almost entirely soluble and assimilable. Their nutitrive value is very high, approximately three times that of fresh meat.[1]

Hayduck[2] has found that yeast taken from the bottom of a fermented liquid contains an appreciable amount of alcohol, which, under the conditions ordinarily prevailing in breweries, amounts to about 3% of the total yeast. As soon as methods for the separation of this alcohol are perfected, it will constitute an important by-product of the yeast-drving process.

ERRATA IN VOL. I.

Page 205, "E. Schlighting" should be "E. Schlichting. 12th line "thoughout" should "be throughout."

Page 207, 9th line, "bodies or peptones" should be "bodies such as peptones."

[1] Hayduck, *Jaabuch Vers. und Lehranst. Brau.*, 1911, 286.
[2] *Jahrb. Vers. Lehranst. Brau.*, 1913, 536.

NEUTRAL ALCOHOLIC DERIVATIVES.

For a comprehensive review of the methods dealing with ether, ethyl chloride and chloroform and of the literature reference should be made as follows:

Ether.—Baskerville and Hamor, *J. Ind. Eng. Chem.*, 3, 301–317.

Ethyl Chloride.—Baskerville and Hamor, *J. Ind. Eng. Chem.*, 5, 828–831.

Chloroform.—Baskerville and Hamor, *J. Ind. Eng. Chem.*, 4, 212–220.

SUGARS.

By WILLIAM A. DAVIS.

Solution Densities.—Probably the most accurate values of the divisors at different concentrations for saccharose, dextrose, lævulose, invert sugar, maltose and "low," "medium" and "high" starch conversion products are those of Brown, Morris and Millar.[1] These authors give the sp. gr. taken at 15.5° and referred to water at the same temperature; the divisors are not exactly grams per *true* 100 c.c. but the weight of substance (weighed in air) contained in a volume of the solution equal to that occupied by 100 grm. of water at 15.5° weighed in air against brass weights. In order to convert the results into grams per true 100 c.c., when great accuracy is desired, as for instance in determining specific rotatory constants, they must be multiplied by the factor 0.99802, thus reducing them by about 0.2%. The following equations summarise the results; in the original paper the results are also given in the form of tables and curves from which the divisors corresponding with different concentrations can be read directly.

D is the required divisor in grams of anhydrous sugar per 100 c.c. (reputed).

G is the sp. gr. at 15.5° when water at the same temperature = 1,000.

For *Dextrose*, $D = 3.848 - 0.00028 (G - 1,000) - 0.0000028 (G - 1,000)^2$.

Lævulose, $D = 3.946 - 0.00068 (G - 1,000) - 0.0000007 (G - 1,000)^2$.

Invert Sugar, $D = 3.897 - 0.00025 (G - 1,000) - 0.0000004 (G - 1,000)^2$.

Maltose, $D = 3.9435 - 0.00044 (G - 1,000) - 0.000001 (G - 1,000)^2$.

"*High Transformation*" of starch by diastase, $[\alpha]_D$ 188.6°, R = 20.2.

$D = 4.032 - 0.0006 (G - 1,000)$.

"*Low Transformation*" of starch, $[\alpha]_D$ 149.7°. R = 82.8.

$D = 3.9742 - 0.000403 (G - 1,000) - 0.0000014 (G - 1,000)^2$.

Ling, Eynon and Lane[2] have since re-determined the solution densities of dextrose, lævulose and maltose, for concentrations ranging from c = 1 to c = 24. The results practically confirm those of Brown, Morris and Millar and are of importance because special care was taken to ensure the purity of the sugars employed. The writer also has made a series of determinations with highly purified dextrose, lævulose, cane sugar and maltose; for all practical purposes the values referred to above may be taken as accurate.[3]

[1] *Trans.*, 1897, 71, 72.
[2] *Seventh Int. Congr. Appl. Chem.*, 1910, 1, 137.
[3] Compare Davis and Daish, *J. Agric., Sci.*, 1913, 5, 437.

Below are given the recent data obtained by Ling, Eynon and Lane.

Column (A) gives the weight of dry substance taken.

Column (B) gives the total weight of solution.

Column (C) gives the sp. gr. of the solution at 15.5°, referred to water at the same temperature.

Column (D) gives the grm. of sugar per 100 c.c. (reputed) (fluid grm. at 15.5°).

Column (E) gives the divisor for calculating grm. of sugar per 100 c.c. (reputed) from the sp. gr.

DEXTROSE.

A	B	C	D	E
0.9992	48.7130	1,007.97	2.0675	3.855
1.0034	48.1702	1,008.08	2.0999	3.848
1.9994	50.4500	1,015.45	4.0243	3.839
3.0003	51.4028	1,022.90	5.9705	3.836
4.0176	51.5618	1,030.72	8.0311	3.825
4.9977	51.8332	1,038.26	10.0108	3.822
5.9990	52.5148	1,045.61	11.9444	3.819
7.0012	52.6845	1,053.38	13.9984	3.813
7.9990	52.8802	1,061.13	16.0513	3.808
9.0015	53.3225	1,068.63	18.0398	3.804
9.9864	53.6794	1,076.03	20.0182	3.798
10.002	53.8410	1,084.06	22.1483	3.795
10.123	53.3030	1,092.42	24.4141	3.786

LÆVULOSE.

A	B	C	D	E
1.1040	51.2864	1,008.55	2.1710	3.938
2.0121	50.9296	1,015.76	4.0130	3.927
3.0125	51.3828	1,023.54	6.0008	3.923
4.0528	52.7696	1,031.05	7.9186	3.921
3.9982	51.5144	1,031.36	8.0047	3.918
5.0020	51.9567	1,039.18	10.0044	3.916
5.9136	52.7690	1,048.83	11.7202	3.910
6.9985	52.6476	1,054.73	14.0207	3.904
8.2876	54.7342	1,062.78	16.0922	3.901
8.9952	53.2702	1,070.43	18.0753	3.896
10.0312	54.0708	1,077.80	19.9949	3.890
11.0257	54.3534	1,085.57	22.0211	3.886
11.6614	52.9094	1,093.55	24.1021	3.881

MALTOSE.

A .	B	C	D	E
0.9422	50.1806	1,007.46	1.8916	3.944
1.9977	50.9160	1,015.66	3.9850	3.930
3.1074	51.3990	1,024.31	6.1926	3.926
3.8939	51.4710	1,030.54	7.7963	3.917
5.0569	52.2945	1,039.34	10.0504	3.914
5.9907	53.6860	1,045.63	11.6680	3.911
7.0032	52.8330	1,054.63	13.9795	3.908
7.9710	53.4580	1,061.78	15.8319	3.902
8.0323	52.9998	1,062.87	16.1081	3.903
8.9098	53.2972	1,069.84	17.8847	3.905
8.9132	53.2600	1,069.91	17.9052	3.904
10.0734	53.7766	1,078.76	20.2073	3.898
10.9333	53.5393	1,086.47	22.1869	3.897
11.7828	54.2558	1,092.35	23.7227	3.893

For tables showing the sp. gr. of saccharose solutions at 20° compared with water at 4° as determined by the *Kaiserliche Normal Eichungskommission*, see *Zeit. Ver. Deutsch. Zuckerind.*, 1900, page 1123. These tables are given in full in Frühling's *Anleitung für die Zuckerindustrie*, 7th Ed., 1911, pages 87–91.

Refractometer Values.—During the past few years the refractometer has found increasing application in sugar analysis and the opinion is growing that the refractometer is an indispensable instrument in all beet cultivation or sugar factory laboratories.[1] For recent tables of refractometric values see Main,[2] and Stanek.[3] Schönrock gives the following values which were determined in the laboratories of the Physikalische-technische Reichsanstalt, Berlin.

REFRACTIVE INDEX AND WATER CONTENT OF SUGAR SOLUTIONS.

$n_D^{20°}$	w	$n_D^{20°}$	w	$n_D^{20°}$	w	$h_D^{20°}$	w
1.3330	100	1.3590	83	1.3883	66	1.4221	49
1.3344	99	1.3606	82	1.3902	65	1.4242	48
1.3359	98	1.3622	81	1.3920	64	1.4264	47
1.3374	97	1.3639	80	1.3939	63	1.4285	46
1.3388	96	1.3655	79	1.3958	62	1.4307	45
1.3403	95	1.3672	78	1.3978	61	1.4329	44
1.3418	94	1.3689	77	1.3997	60	1.4351	43
1.3433	93	1.3706	76	1.4016	59	1.4373	42
1.3448	92	1.3723	75	1.4036	58	1.4396	41
1.3464	91	1.3740	74	1.4056	57	1.4418	40
1.3479	90	1.3758	73	1.4076	56	1.4441	39
1.3494	89	1.3775	72	1.4096	55	1.4464	38
1.3510	88	1.3793	71	1.4117	54	1.4486	37
1.3526	87	1.3811	70	1.4137	53	1.4509	36
1.3541	86	1.3829	69	1.4158	52	1.4532	35
1.3557	85	1.3847	68	1.4179	51	1.4555	34
1.3573	84	1.3865	67	1.4200	50		

Relationship of Polarimetric Readings in Different Instruments.—The following data for converting the readings obtained with different types of polarimeter are given by Brown, Morris and Millar.[4] The relationship of the readings obtained with a sodium light instrument to those obtained with a Ventzke-Scheibler instrument varies slightly according to the substance under observation and its concentration. The table given is therefore of considerable use in working with the different sugars.

Column A gives for each solution the result of dividing the scale reading of the Ventzke-Scheibler instrument by the circle reading of a Jellett-Cornu scale; it gives therefore the number of V.-S. scale divisions corresponding to 1° of the sodium-light scale. Column B gives the value of a scale division of the V.-S. instrument in terms of ray D.

Column C gives for each instrument the ratio of $[\alpha]_j$ Biot to $[\alpha]_D$.

Column D gives the ratio of $[\alpha]_j$, Montgolfier to $[\alpha]_D$. For a discussion of

[1] Compare Pellet, *Int. Sugar J.*, 1914, 16, 521.
[2] *Int. Sugar J.*, 9, 481.
[3] *Zeit. Ver. Deutsch. Zuckerind.*, 61, 421.
[4] *Trans.*, 1897, 71, 93.

the relationship existing in each case between [α]ⱼ Biot (Biot's *jaune moyen*) and [α]ⱼ Montgolfier see Brown, Morris and Millar (*loc. cit.*).

	Concentration.	A	B	C	D
Saccharose................	10 %	2.882	0.3469	1.107	1.130
Maltose..................	10 %	2.899	0.3449	1.113	1.136
Maltose..................	5 %	2.892	0.3457	1.111	1.134
Dextrose.................	10 %	2.904	0.3442	1.115	1.138
Dextrose.................	5 %	2.894	0.3454	1.111	1.134
Starch products..........	10 %	2.891	0.3458	1.111	1.134
Starch products..........	5 %	2.895	0.3454	1.111	1.134

General Methods.—Certain sources of error in estimating sugars by gravimetric and volumetric methods are dealt with in a paper by Davis and Daish.[1] In the gravimetric method, in which the precipitate of cuprous oxide obtained is collected on asbestos, the necessity of previously digesting the asbestos with boiling 20% sodium hydroxide solution and subsequently thoroughly washing with water is emphasised; unless the asbestos is treated in this way considerable loss of weight may occur owing to the action of the hot Fehling solution on impurities present in the asbestos. It is probably best and simplest to collect the cuprous oxide precipitate in a Gooch crucible containing a layer ¼ to ½ in. thick of the purified asbestos and, after thoroughly washing with boiling water, to wash with a little alcohol and ether and dry in a steam oven. The Gooch crucible is then placed in an ordinary No. 1 Berlin crucible (which serves to shield it from direct contact with the flame) and is heated strongly over a ½-in. Teclu or Fletcher Argand gas flame for ½ hour; it is then allowed to cool in the desiccator at least 1 hour, weighed and again heated for another 30 minutes. The weight is generally practically constant after the first heating, the increase of weight in the second heating seldom exceeding 0.0005 grm. If the Gooch crucible is shielded from direct contact with the flame in the way indicated there is never any difficulty in obtaining accurate results for the weight of cupric oxide and the process is far more simple and rapid than collecting the cuprous oxide in a Soxhlet tube and reducing to copper in a stream of hydrogen in the manner generally advocated. The same crucible and asbestos can be used over and over again for 10 to 20 successive charges without dissolving away the cupric oxide; the fresh charge of cuprous oxide is collected on the top of the previous charge of cupric oxide. It is only necessary to ensure that this is constant in weight. Elion[2] and others have stated that conversion to cupric oxide gives unreliable and discordant results, but this is not true when the cupric oxide is shielded from the reducing gases of the flame. Under the conditions given the ratio of $\dfrac{2CuO}{Cu_2O}$ is found to be 1.111 to 1.112 the theoretical ratio (Cu = 63.57) being 1.112. H. Pellet (*Private*

[1] *J. Agric. Science*, 1913, **5**, 437.
[2] *Zeit. angew. Chem.*, 1890, 325.

Communication) recommends igniting the cuprous oxide precipitate by heating it *at not too high a temperature* in a muffle furnace. A blowpipe should never be used, even when the Gooch crucible is shielded by an outer crucible, as low results are then obtained, probably owing to the slight dissociation of cupric oxide which occurs at very high temperatures: the ratio $\dfrac{2CuO}{Cu_2O}$ when the blowpipe is used ranges from 1.105 to 1.109 instead of having practically the theoretical value 1.112 (Davis and Daish, *loc. cit.*).

The recommendation is frequently made to weigh the cuprous oxide, as such, after drying at 100° and this method has been prescribed by the U. S. Bureau of Chemistry[1] as one of its provisional methods. Whilst this course is quite safe in the case of pure sugars it involves considerable error when dealing with impure solutions containing organic substances such as are obtained when working with plant or animal extracts, even when these have been partially purified by treatment with basic lead acetate, etc.; the same is true of the solutions obtained on inverting or hydrolysing starch or sugars by enzyme preparations, such as diastase, maltase or invertase, or after fermenting sugars by yeasts, even though alumina cream is subsequently used to clear the solutions. In all such cases, the cuprous oxide invariably contains organic matter, which burns away during ignition, so that the ratio $\dfrac{CuO}{Cu_2O}$ is thereby diminished; this ratio varies from 1.060 to 1.105, according to the nature of the solution and of the enzyme preparation employed. It is probable that in dealing with yeasts, invertase, etc., the cuprous oxide precipitate contains traces of copper compounds of amino-acids, proteins, etc., as well as colloidal matter carried down by adsorption. In such cases the cupric oxide weighed would be slightly higher than that actually due to reduction only; but numerous experiments indicate that this error is relatively small and not likely to interfere with the results obtained. An alternative method of procedure in such cases is to estimate the actual copper present in the cuprous oxide precipitate by one of the standard volumetric methods; the A. O. A. C. have recently adopted as a provisional process ·Low's thiosulphate method (see below, page 41). H. Pellet[2] advocates the use of potassium cyanide (Parker's method) under the conditions worked out by C. Müller.[3]

The method which depends on using ferric sulphate to dissolve the cuprous oxide and subsequently titrating back with permanganate, is criticised by Davis and Daish (*loc. cit.*) who came to the conclusion that it cannot be regarded as one of the most accurate methods for the purpose. This method has, however, been widely used in recent years and has been adopted as provisional by the A. O. A. C.[4] In biochemical work it has been extensively

[1] *Bulletin* 107 (revised), 1912, page 53. Allen Vol. I, page 325.
[2] *Bull. Assoc. Chem. Sucr.*, 1914, 915.
[3] *Bull. Assoc. Chem. Sucr.*, 1911–1912, page 71.
[4] *Bulletin* 107 (revised), 1912, page 52. Allen, Vol. I, page 324.

employed since Bertrand advocated its use[1] and gave tables for dextrose, invert sugar, maltose and lactose. Davis and Daish state that in their hands this method gave values from 1 to 1.5% in error for pure dextrose and maltose and point out that the constants given by Bertrand for his dextrose ($[\alpha]_D = 52.0°$) and maltose (137.4°) are not those of sugars of the highest degree of purity. In preparing the solution of invert sugar on which his tables are based, Bertrand hydrolysed cane sugar by heating with 2% hydrochloric acid for 10 to 15 minutes at 100°; such treatment invariably causes slight destruction of lævulose and Davis and Daish in consequence found results by using Bertrand's tables which were 3 to 5% low. That decomposition of invert sugar occurs with dilute hydrochloric acid at temperatures above 70° has been generally recognised since the work of Herzfeld.[2] The volumetric method using permanganate is, too, not so advantageous as the method of Ling, Rendle and Jones (see below, page 38) either on the ground of rapidity (for which it is generally preferred to gravimetric methods) or of accuracy. It is to be regarded only as a fairly rapid approximate method which may prove useful when no high degree of accuracy is required.

Gravimetric Methods for Reducing Sugars.—Probably the most accurate method of estimating the reducing sugars (dextrose, lævulose, invert sugar and maltose) is to work under the conditions laid down by Brown, Morris and Millar[3] employing the tables they have given for these sugars. Davis and Daish using highly purified specimens of the sugars verified these tables and found a quite satisfactory agreement. The probable error in the copper oxide weighed under these conditions is not likely to be more than 1 mg., which, when 0.20 to 0.40 grm. is actually obtained, gives an error well within 0.5%. It is certainly preferable in the majority of cases to work under the well-defined conditions laid down by Brown, Morris and Millar than to use Allihn's method, in which the size of the beaker, the kind and height of the flame, undoubtedly influence the results; the shortness of the time of heating is, too, a disadvantage, as any slight differences thus exercise proportionately greater effect. The tables given by Wein for maltose, which have been very generally used (see Allen, Vol. I, page 363) and have been provisionally adopted in the United States (*Bulletin* 107 *revised*) were shown by Brown, Morris and Millar in 1897 to give results 5% low; Ling and Baker[4] confirmed this.

Brown, Morris and Millar's Method.—This is a modification of the gravimetric process suggested by O'Sullivan[5] the essential point being that regular heating is ensured by immersing the beaker during the reduction in boiling water; the time of heating is 12 minutes. The solutions used are as follows:

[1] *Bull. Soc. Chim.*, 1906 [iii], **35**, 1285.
[2] *Zeit. Ver. Zuck. Ind.*, 1898, 699 and 742.
[3] *Trans.*, 1897, **71**, 105.
[4] *Trans.*, 1897, **71**, 509.
[5] *Trans.*, 1876, **30**, 150.

A. Recrystallised copper sulphate 34.6 grm.
 Water to 500 c.c.
B. Rochelle salt 173.0 grm.
 Anhydrous sodium hydroxide 65.0 grm.
 Water to 500 c.c.

As the oxidising power of the solution is very sensitive to small changes in the amount of alkali present great care must be taken to ensure accuracy in the weight of sodium hydroxide. This is best done by preparing a somewhat concentrated solution of sodium hydroxide (free from carbonate), ascertaining the strength by means of the density, and then diluting so that a known volume (300 c.c. for example) represents exactly 65.0 grm. It is convenient to prepare large volumes (7 to 10 litres) of each of the solutions A and B and to store these in large glass bottles (Welsbach bottles) so arranged as to feed two 25 c.c. automatic pipettes. Access of carbon dioxide of the air to solution B should be guarded against by a U-tube containing soda-lime.

25 c.c. of each solution A and B are introduced into a beaker, the surface area of which[1] is 44 sq. cm.; the beaker is covered with a clock glass and immersed in a bath of *boiling* water until the temperature of the solution is the same as that of the bath. This is usually the case in 6 minutes. The sugar solution is now added and the total volume diluted if necessary to 100 c.c.; it is best if possible to have the sugar solution of such a concentration that 50 c.c. can be taken directly for the estimation. If the sugar solution is considerably more concentrated, 25 c.c. (or 30 c.c.) are taken and a corresponding quantity of boiling water (25 c.c. or 20 c.c.) added. The beaker is then immediately covered with the clock glass and heated exactly 12 minutes in boiling water; the precipitated cuprous oxide is then filtered as rapidly as possible through a Soxhlet tube or preferably a Gooch crucible, thoroughly washed with about 400 c.c. of boiling water, dried and weighed, either as cupric oxide (ignition as above) or after reduction to metallic copper.

The amount of reducing sugar taken for an estimation should give a weight of cupric oxide lying within the limits 0.15 to 0.40 grm. Tables I and II give the relationship existing between the weight of copper (or of CuO) and the various sugars.

A convenient heating bath is shown in Fig. 1 for use with the beaker flasks employed in the reduction (Davis and Daish, *loc. cit.*). It consists of a 10-in. enamelled iron saucepan, 4½ in. deep, into which a false bottom of copper plate is placed so as to afford a convenient support for the beaker flasks. The cover of the bath is made of copper and consists of two halves each perforated with two 2½-in. holes, the edge of the plate being

[1] A Jena beaker flask of 250 c.c. can be conveniently used here; such vessels, with a top diameter 2¼ in., bottom diameter 2⅜ in., give accurately the results contained in Brown. Morris and Millar's tables (Davis and Daish, *loc. cit.*), and are far more convenient in manipulation than ordinary beakers. Pellet also (*Bull. Assoc. Chem. Sucr.*, 1913, 198) has independently suggested the use of beaker flasks for measurements of the reducing power of sugars.

turned down so as to fit over the bath.   Each half of the cover can be lifted
off separately so as to admit the beaker flask containing the Fehling solution;
with this arrangement several beaker-flasks can be heated simultaneously.

The most carefully prepared Fehling solution usually gives a very slight
precipitate of cuprous oxide on heating, owing to spontaneous reduction;
this value should be determined for every fresh batch of solution and be
allowed for in the final result of each determination.   It usually amounts to
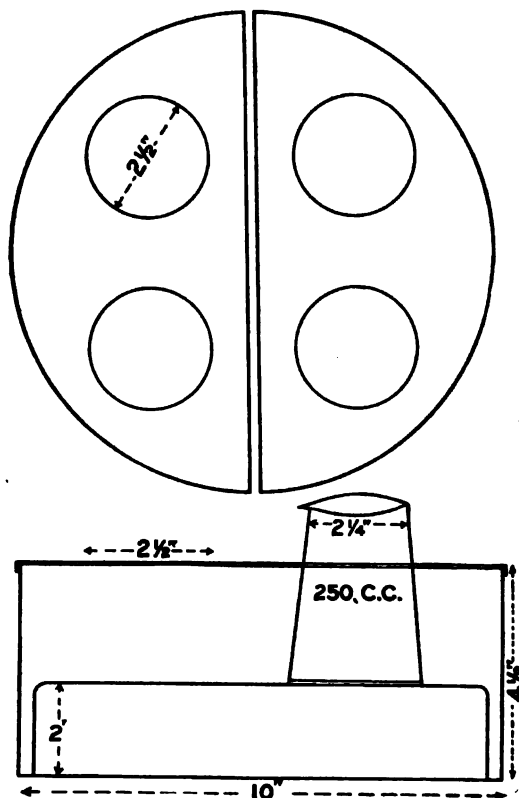
Fig. 1.

o.oo2 to o.oo3 grm. per 50 c.c. of Fehling solution.   The writer finds that
when the two solutions A and B after being prepared are separately stored
in stoppered bottles for a week or fortnight before use a considerable quantity
of sediment separates from each; when the solutions are filtered and then
tested in the usual way, in a "blank" experiment, the amount of self-reduc-
tion is very small, usually o.oooo to o.oo1o grm. per 50 c.c. of solution.   The
"self-reduction" thus rapidly diminishes immediately after the solutions are
made up, so that it is always advisable to store the solutions for at least a
fortnight before use.

TABLE I.—REDUCING POWER OF DEXTROSE, LÆVULOSE AND INVERT SUGAR UNDER BROWN, MORRIS AND MILLAR'S CONDITIONS.

Sugar, mg.	Dextrose			Lævulose			Invert sugar		
	Cu, grm.	CuO, grm.	CuO corresponding to 1 grm.	Cu, grm.	CuO, grm.	CuO corresponding to 1 grm.	Cu, grm.	CuO, grm.	CuO corresponding to 1 grm.
50	0.1030	0.1289	2.578	0.0923	0.1155	2.340	0.0975	0.1221	2.442
55	0.1134	0.1422	2.585	0.1027	0.1287	2.341	0.1076	0.1349	2.453
60	0.1238	0.1552	2.587	0.1122	0.1407	2.345	0.1176	0.1474	2.457
65	0.1342	0.1682	2.589	0.1216	0.1524	2.346	0.1275	0.1598	2.459
70	0.1443	0.1809	2.585	0.1312	0.1645	2.350	0.1373	0.1721	2.459
75	0.1543	0.1935	2.580	0.1405	0.1761	2.349	0.1468	0.1840	2.454
80	0.1644	0.2061	2.577	0.1500	0.1881	2.351	0.1566	0.1963	2.454
85	0.1740	0.2187	2.572	0.1590	0.1993	2.345	0.1662	0.2084	2.451
90	0.1834	0.2299	2.555	0.1686	0.2114	2.349	0.1755	0.2200	2.445
95	0.1930	0.2420	2.547	0.1774	0.2224	2.341	0.1848	0.2317	2.439
100	0.2027	0.2538	2.538	0.1862	0.2331	2.331	0.1941	0.2430	2.430
105	0.2123	0.2662	2.535	0.1952	0.2447	2.331	0.2034	0.2550	2.429
110	0.2218	0.2781	2.528	0.2040	0.2558	2.325	0.2128	0.2668	2.425
115	0.2313	0.2900	2.522	0.2129	0.2669	2.321	0.2220	0.2783	2.420
120	0.2404	0.3014	2.512	0.2215	0.2777	2.314	0.2311	0.2898	2.415
125	0.2496	0.3130	2.504	0.2303	0.2887	2.310	0.2400	0.3009	2.407
130	0.2585	0.3241	2.493	0.2390	0.2997	2.305	0.2489	0.3121	2.400
135	0.2675	0.3354	2.484	0.2477	0.3106	2.300	0.2578	0.3232	2.394
140	0.2762	0.3463	2.473	0.2559	0.3209	2.292	0.2663	0.3339	2.385
145	0.2850	0.3573	2.464	0.2641	0.3311	2.284	0.2750	0.3448	2.378
150	0.2934	0.3673	2.448	0.2723	0.3409	2.273	0.2832	0.3546	2.364
155	0.3020	0.3787	2.443	0.2805	0.3517	2.269	0.2915	0.3655	2.358
160	0.3103	0.3891	2.432	0.2889	0.3622	2.264	0.3002	0.3764	2.352
165	0.3187	0.3996	2.422	0.2972	0.3726	2.258	0.3086	0.3869	2.345
170	0.3268	0.4098	2.410	0.3053	0.3828	2.252	0.3167	0.3971	2.336
175	0.3350	0.4200	2.400	0.3134	0.3930	2.245	0.3251	0.4076	2.329
180	0.3431	0.4302	2.390	0.3216	0.4032	2.240	0.3331	0.4177	2.320
185	0.3508	0.4399	2.377	0.3297	0.4134	2.234	0.3410	0.4276	2.311
190	0.3590	0.4501	2.369	0.3377	0.4234	2.228	0.3490	0.4376	2.303
195	0.3668	0.4599	2.358	0.3457	0.4335	2.223	0.3570	0.4476	2.295
200	0.3745	0.4689	2.344	0.3539	0.4431	2.216	0.3650	0.4570	2.285
205	0.3822	0.4792	2.338	0.3616	0.4534	2.211	0.3726	0.4672	2.279

TABLE II.—REDUCING POWER OF MALTOSE UNDER BROWN, MORRIS AND MILLAR'S CONDITIONS.

Maltose, mg.	Cu weighed, grm.	CuO weighed, grm.	CuO corresponding to 1 grm. maltose	Maltose, mg.	Cu weighed, grm.	CuO weighed, grm.	CuO corresponding to 1 grm. maltose
70	0.0772	0.0966	1.380	190	0.2072	0.2593	1.371
75	0.0826	0.1034	1.380	195	0.2126	0.2661	1.370
80	0.0880	0.1102	1.379	200	0.2180	0.2729	1.370
85	0.0934	0.1169	1.379	205	0.2234	0.2797	1.370
90	0.0988	0.1237	1.378	210	0.2288	0.2865	1.369
95	0.1042	0.1305	1.378	215	0.2342	0.2933	1.369
100	0.1097	0.1373	1.378	220	0.2397	0.3000	1.3685
105	0.1151	0.1441	1.377	225	0.2451	0.3068	1.368
110	0.1205	0.1509	1.377	230	0.2505	0.3136	1.368
115	0.1259	0.1576	1.3765	235	0.2559	0.3203	1.367
120	0.1313	0.1644	1.376	240	0.2613	0.3272	1.367
125	0.1367	0.1712	1.376	245	0.2667	0.3340	1.3665
130	0.1422	0.1779	1.375	250	0.2722	0.3407	1.366
135	0.1476	0.1848	1.375	255	0.2776	0.3475	1.366
140	0.1530	0.1916	1.375	260	0.2830	0.3543	1.365
145	0.1584	0.1983	1.374	265	0.2884	0.3610	1.365
150	0.1634	0.2051	1.374	270	0.2938	0.3678	1.365
155	0.1692	0.2119	1.373	275	0.2992	0.3747	1.364
160	0.1747	0.2186	1.373	280	0.3047	0.3814	1.364
165	0.1801	0.2254	1.373	285	0.3101	0.3882	1.3635
170	0.1855	0.2323	1.372	290	0.3155	0.3950	1.363
175	0.1909	0.2390	1.372	295	0.3209	0.4017	1.363
180	0.1963	0.2458	1.3715	300	0.3264	0.4085	1.362
185	0.2017	0.2526	1.371	305	0.3318	0.4154	1.362

PROVISIONAL UNITED STATES A. O. A. C. GRAVIMETRIC METHOD FOR REDUCING SUGARS.

The following are the conditions which have been adopted as provisional by the A. O. A. C.,[1] the tables are due to Munson and Walker.[2]

(1) Preparation of Solutions and Asbestos.

(a) Solutions.—Use the solutions in Vol. I, page 318, under Soxhlet's modification of Fehling's solution.[3]

(b) Asbestos.—Prepare the asbestos, which should be the amphibole variety, by first digesting with 1:3 hydrochloric acid for 2 or 3 days. Wash free from acid and digest for a similar period with sodium hydroxide solution, after which treat for a few hours with hot alkaline copper tartrate solution of the strength employed in sugar determinations. Then wash the asbestos free from alkali, finally digest with nitric acid for several hours and after washing free from acid shake with water for use. In preparing the Gooch crucible load it with a film of asbestos ¼ in. thick, wash this thoroughly with water to remove fine particles of asbestos; finally wash with alcohol and ether, dry for 30 minutes at 100° C., cool in a desiccator and weigh. It is best to dissolve the cuprous oxide with nitric acid each time after weighing and use the same felts over and over again, as they improve with use.

(2) Determination.

Transfer 25 c.c. each of the copper and alkaline tartrate solutions to a 400 c.c. Jena or Non-sol beaker and add 50 c.c. of reducing sugar solution, or, if a smaller volume of sugar solution be used, add water to make the final volume 100 c.c. Heat the beaker upon an asbestos gauze over a Bunsen burner, so regulate the flame that boiling begins in 4 minutes, and continue the boiling for exactly 2 minutes. Keep the beaker covered with a watch-glass throughout the entire time of heating. Without diluting, filter the cuprous oxide at once on an asbestos felt in a porcelain Gooch crucible, using suction. Wash the cuprous oxide thoroughly with water at a temperature of about 60° C., then with 10 c.c. of alcohol and finally with 10 c.c. of ether. Dry for 30 minutes in a water oven at 100° C., cool in a desiccator and *weigh as cuprous oxide.*

N. B. The number of milligrams of copper reduced by a given amount of reducing sugar differs when saccharose is present and when it is absent. In the tables following, the absence of saccharose is assumed except in the two columns under invert sugar, where one for mixtures of invert sugar·and saccharose (0.4 grm. of total sugar in 50 c.c. of solution) and one for invert sugar and saccharose when the 50 c.c. of solution contains 2 grm. of total sugar are given, in addition to the column for invert sugar alone.

[1] *Bulletin* 107 (*revised*), 1912, page 241.
[2] *J. Amer. Chem. Soc.*, 1906, 28, 663; 1907, 29, 541.
[3] The tartrate solution used by the A. O. A. C. contains 173 grm. of Rochelle salt, and 50 grm. of sodium hydroxide in 500 c.c.

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR IN THE PRESENCE OF SACCHAROSE (0.4 GRM. AND 2 GRM. TOTAL SUGAR), LACTOSE (THREE FORMS), AND MALTOSE (ANHYDROUS AND CRYSTALLISED).

(For Lactose Figures see pages 58 to 61.)

(Expressed in milligrams.)

Cuprous oxide (Cu₂O)	Copper (Cu)	Dextrose (d-glucose)	Invert sugar	Invert sugar and saccharose		Maltose		Cuprous oxide (Cu₂O)
				0.4 grm. total sugar	2 grm. total sugar	$C_{12}H_{22}O_{11}$	$C_{12}H_{22}O_{11} + H_2O$	
10	8.9	4.0	4.5	1.6	....	5.9	6.2	10
11	9.8	4.5	5.0	2.1	....	6.7	7.0	11
12	10.7	4.9	5.4	2.5	....	7.5	7.9	12
13	11.5	5.3	5.8	3.0	....	8.3	8.7	13
14	12.4	5.7	6.3	3.4	....	9.1	9.5	14
15	13.3	6.2	6.7	3.9	.	9.9	10.4	15
16	14.2	6.6	7.2	4.3	....	10.6	11.2	16
17	15.1	7.0	7.6	4.8	....	11.4	12.0	17
18	16.0	7.5	8.1	5.2	....	12.2	12.9	18
19	16.9	7.9	8.5	5.7	....	13.0	13.7	19
20	17.8	8.3	8.9	6.1	....	13.8	14.6	20
21	18.7	8.7	9.4	6.6	....	14.6	15.4	21
22	19.5	9.2	9.8	7.0	....	15.4	16.2	22
23	20.4	9.6	10.3	7.5	....	16.2	17.1	23
24	21.3	10.0	10.7	7.9	....	17.0	17.9	24
25	22.2	10.5	11.2	8.4	....	17.8	18.7	25
26	23.1	10.9	11.6	8.8	....	18.6	19.6	26
27	24.0	11.3	12.0	9.3	....	19.4	20.4	27
28	24.9	11.8	12.5	9.7	....	20.2	21.2	28
29	25.8	12.2	12.9	10.2	....	21.0	22.1	29
30	26.6	12.6	13.4	10.7	4.3	21.8	22.9	30
31	27.5	13.1	13.8	11.1	4.7	22.6	23.7	31
32	28.4	13.5	14.3	11.6	5.2	23.3	24.6	32
33	29.3	13.9	14.7	12.0	5.6	24.1	25.4	33
34	30.2	14.3	15.2	12.5	6.1	24.9	26.2	34
35	31.1	14.8	15.6	12.9	6.5	25.7	27.1	35
36	32.0	15.2	16.1	13.4	7.0	26.5	27.9	36
37	32.9	15.6	16.5	13.8	7.4	27.3	28.7	37
38	33.8	16.1	16.9	14.3	7.9	28.1	29.6	38
39	34.6	16.5	17.4	14.7	8.4	28.9	30.4	39
40	35.5	16.9	17.8	15.2	8.8	29.7	31.3	40
41	36.4	17.4	18.3	15.6	9.3	30.5	32.1	41
42	37.3	17.8	18.7	16.1	9.7	31.3	32.9	42
43	38.2	18.2	19.2	16.6	10.2	32.1	33.8	43
44	39.1	18.7	19.6	17.0	10.7	32.9	34.6	44
45	40.0	19.1	20.1	17.5	11.1	33.7	35.4	45
46	40.9	19.6	20.5	17.9	11.6	34.4	36.3	46
47	41.7	20.0	21.0	18.4	12.0	35.2	37.1	47
48	42.6	20.4	21.4	18.8	12.5	36.0	37.9	48
49	43.5	20.9	21.9	19.3	12.9	36.8	38.8	49
50	44.4	21.3	22.3	19.7	13.4	37.6	39.6	50
51	45.3	21.7	22.8	20.2	13.9	38.4	40.4	51
52	46.2	22.2	23.2	20.7	14.3	39.2	41.3	52
53	47.1	22.6	23.7	21.1	14.8	40.0	42.1	53
54	48.0	23.0	24.1	21.6	15.2	40.8	42.9	54
55	48.9	23.5	24.6	22.0	15.7	41.6	43.8	55
56	49.7	23.9	25.0	22.5	16.2	42.4	44.6	56
57	50.6	24.3	25.5	22.9	16.6	43.2	45.4	57
58	51.5	24.8	25.9	23.4	17.1	44.0	46.3	58
59	52.4	25.2	26.4	23.9	17.5	44.8	47.1	59
60	53.3	25.6	26.8	24.3	18.0	45.6	48.0	60
61	54.2	26.1	27.3	24.8	18.5	46.3	48.8	61
62	55.1	26.5	27.7	25.2	18.9	47.1	49.6	62
63	56.0	27.0	28.2	25.7	19.4	47.9	50.5	63
64	56.8	27.4	28.6	26.2	19.8	48.7	51.3	64

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR IN THE PRESENCE OF SACCHAROSE (0.4 GRM. AND 2 GRM. TOTAL SUGAR), LACTOSE (THREE FORMS), AND MALTOSE (ANHYDROUS AND CRYSTALLISED).

(For Lactose Figures see pages 58 to 61.)—*Continued.*

(Expressed in milligrams.)

Cuprous oxide (Cu₂O)	Copper (Cu)	Dextrose (d-glucose)	Invert sugar	Invert sugar and saccharose		Maltose		Cuprous oxide (Cu₂O)
				0.4 grm. total sugar	2 grm. total sugar	$C_{12}H_{22}O_{11}$	$C_{12}H_{22}O_{11}$ + H_2O	
65	57.7	27.8	29.1	26.6	20.3	49.5	52.1	65
66	58.6	28.3	29.5	27.1	20.8	50.3	53.0	66
67	59.5	28.7	30.0	27.5	21.2	51.1	53.8	67
68	60.4	29.2	30.4	28.0	21.7	51.9	54.6	68
69	61.3	29.6	30.9	28.5	22.2	52.7	55.5	69
70	62.2	30.0	31.3	28.9	22.6	53.5	56.3	70
71	63.1	30.5	31.8	29.4	23.1	54.3	57.1	71
72	64.0	30.9	32.3	29.8	23.5	55.1	58.0	72
73	64.8	31.4	32.7	30.3	24.0	55.9	58.8	73
74	65.7	31.8	33.2	30.8	24.5	56.7	59.6	74
75	66.6	32.2	33.6	31.2	24.9	57.5	60.5	75
76	67.5	32.7	34.1	31.7	25.4	58.2	61.3	76
77	68.4	33.1	34.5	32.1	25.9	59.0	62.1	77
78	69.3	33.6	35.0	32.6	26.3	59.8	63.0	78
79	70.2	34.0	35.4	33.1	26.8	60.6	63.8	79
80	71.1	34.4	35.9	33.5	27.3	61.4	64.6	80
81	71.9	34.9	36.3	34.0	27.7	62.2	65.5	81
82	72.8	35.3	36.8	34.5	28.2	63.0	66.3	82
83	73.7	35.8	37.3	34.9	28.6	63.8	67.1	83
84	74.6	36.2	37.7	35.4	29.1	64.6	68.0	84
85	75.5	36.7	38.2	35.8	29.6	65.4	68.8	85
86	76.4	37.1	38.6	36.3	30.0	66.2	69.7	86
87	77.3	37.5	39.1	36.8	30.5	67.0	70.5	87
88	78.2	38.0	39.5	37.2	31.0	67.8	71.3	88
89	79.1	38.4	40.0	37.7	31.4	68.5	72.2	89
90	79.9	38.9	40.4	38.2	31.9	69.3	73.0	90
91	80.8	39.3	40.9	38.6	32.4	70.1	73.8	91
92	81.7	39.8	41.4	39.1	32.8	70.9	74.7	92
93	82.6	40.2	41.8	39.6	33.3	71.7	75.5	93
94	83.5	40.6	42.3	40.0	33.8	72.5	76.3	94
95	84.4	41.1	42.7	40.5	34.2	73.3	77.2	95
96	85.3	41.5	43.2	41.0	34.7	74.1	78.0	96
97	86.2	42.0	43.7	41.4	35.2	74.9	78.8	97
98	87.1	42.4	44.1	41.9	35.6	75.7	79.7	98
99	87.9	42.9	44.6	42.4	36.1	76.5	80.5	99
100	88.8	43.3	45.0	42.8	36.6	77.3	81.3	100
101	89.7	43.8	45.5	43.3	37.0	78.1	82.2	101
102	90.6	44.2	46.0	43.8	37.5	78.8	83.0	102
103	91.5	44.7	46.4	44.2	38.0	79.6	83.8	103
104	92.4	45.1	46.9	44.7	38.5	80.4	84.7	104
105	93.3	45.5	47.3	45.2	38.9	81.2	85.5	105
106	94.2	46.0	47.8	45.6	39.4	82.0	86.3	106
107	95.0	46.4	48.3	46.1	39.9	82.8	87.2	107
108	95.9	46.9	48.7	46.6	40.3	83.6	88.0	108
109	96.8	47.3	49.2	47.0	40.8	84.4	88.8	109
110	97.7	47.8	49.6	47.5	41.3	85.2	89.7	110
111	98.6	48.2	50.1	48.0	41.7	86.0	90.5	111
112	99.5	48.7	50.6	48.4	42.2	86.8	91.3	112
113	100.4	49.1	51.0	48.9	42.7	87.6	92.2	113
114	101.3	49.6	51.5	49.4	43.2	88.4	93.0	114
115	102.2	50.0	51.9	49.8	43.6	89.2	93.9	115
116	103.0	50.5	52.4	50.3	44.1	90.0	94.7	116
117	103.9	50.9	52.9	50.8	44.6	90.7	95.5	117
118	104.8	51.4	53.3	51.2	45.0	91.5	96.4	118
119	105.7	51.8	53.8	51.7	45.5	92.3	97.2	119

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR IN THE PRESENCE OF SACCHAROSE (0.4 GRM. AND 2 GRM. TOTAL SUGAR), LACTOSE (THREE FORMS), AND MALTOSE (ANHYDROUS AND CRYSTALLISED).

(For Lactose Figures see pages 58 to 61.)—*Continued*.

(Expressed in milligrams.)

Cuprous oxide (Cu₂O)	Copper (Cu)	Dextrose (d-glucose)	Invert sugar	Invert sugar and saccharose		Maltose		Cuprous oxide (Cu₂O)
				0.4 grm. total sugar	2 grm. total sugar	$C_{12}H_{22}O_{11}$	$C_{12}H_{22}O_{11}$ + H_2O	
120	106.6	52.3	54.3	52.2	46.0	93.1	98.0	120
121	107.5	52.7	54.7	52.7	46.5	93.9	98.9	121
122	108.4	53.2	55.2	53.1	46.9	94.7	99.7	122
123	109.3	53.6	55.7	53.6	47.4	95.5	100.5	123
124	110.1	54.1	56.1	54.1	47.9	96.3	101.4	124
125	111.0	54.5	56.6	54.5	48.3	97.1	102.2	125
126	111.9	55.0	57.0	55.0	48.8	97.9	103.0	126
127	112.8	55.4	57.5	55.5	49.3	98.7	103.9	127
128	113.7	55.9	58.0	55.9	49.8	99.4	104.7	128
129	114.6	56.3	58.4	56.4	50.2	100.2	105.5	129
130	115.5	56.8	58.9	56.9	50.7	101.0	106.4	130
131	116.4	57.2	59.4	57.4	51.2	101.8	107.2	131
132	117.3	57.7	59.8	57.8	51.7	102.6	108.0	132
133	118.1	58.1	60.3	58.3	52.1	103.4	108.9	133
134	119.0	58.6	60.8	58.8	52.6	104.2	109.7	134
135	119.9	59.0	61.2	59.3	53.1	105.0	110.5	135
136	120.8	59.5	61.7	59.7	53.6	105.8	111.4	136
137	121.7	60.0	62.2	60.2	54.0	106.6	112.2	137
138	122.6	60.4	62.6	60.7	54.5	107.4	113.0	138
139	123.5	60.9	63.1	61.2	55.0	108.2	113.9	139
140	124.4	61.3	63.6	61.6	55.5	109.0	114.7	140
141	125.2	61.8	64.0	62.1	55.9	109.8	115.5	141
142	126.1	62.2	64.5	62.6	56.4	110.5	116.4	142
143	127.0	62.7	65.0	63.1	56.9	111.3	117.2	143
144	127.9	63.1	65.4	63.5	57.4	112.1	118.0	144
145	128.8	63.6	65.9	64.0	57.8	112.9	118.9	145
146	129.7	64.0	66.4	64.5	58.3	113.7	119.7	146
147	130.6	64.5	66.9	65.0	58.8	114.5	120.5	147
148	131.5	65.0	67.3	65.4	59.3	115.3	121.4	148
149	132.4	65.4	67.8	65.9	59.7	116.1	122.2	149
150	133.2	65.9	68.3	66.4	60.2	116.9	123.0	150
151	134.1	66.3	68.7	66.9	60.7	117.7	123.9	151
152	135.0	66.8	69.2	67.3	61.2	118.5	124.7	152
153	135.9	67.2	69.7	67.8	61.7	119.3	125.5	153
154	136.8	67.7	70.1	68.3	62.1	120.0	126.4	154
155	137.7	68.2	70.6	68.8	62.6	120.8	127.2	155
156	138.6	68.6	71.1	69.2	63.1	121.6	128.0	156
157	139.5	69.1	71.6	69.7	63.6	122.4	128.9	157
158	140.3	69.5	72.0	70.2	64.1	123.2	129.7	158
159	141.2	70.0	72.5	70.7	64.5	124.0	130.5	159
160	142.1	70.4	73.0	71.2	65.0	124.8	131.4	160
161	143.0	70.9	73.4	71.6	65.5	125.6	132.2	161
162	143.9	71.4	73.9	72.1	66.0	126.4	133.0	162
163	144.8	71.8	74.4	72.6	66.5	127.2	133.9	163
164	145.7	72.3	74.9	73.1	66.9	128.0	134.7	164
165	146.6	72.8	75.3	73.6	67.4	128.8	135.5	165
166	147.5	73.2	75.8	74.0	67.9	129.6	136.4	166
167	148.3	73.7	76.3	74.5	68.4	130.3	137.2	167
168	149.2	74.1	76.8	75.0	68.9	131.1	138.0	168
169	150.1	74.6	77.2	75.5	69.3	131.9	138.9	169
170	151.0	75.1	77.7	76.0	69.8	132.7	139.7	170
171	151.9	75.5	78.2	76.4	70.3	133.5	140.5	171
172	152.8	76.0	78.7	76.9	70.8	134.3	141.4	172
173	153.7	76.4	79.1	77.4	71.3	135.1	142.2	173
174	154.6	76.9	79.6	77.9	71.7	135.9	143.0	174

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR IN THE PRESENCE OF SACCHAROSE (0.4 GRM. AND 2 GRM. TOTAL SUGAR), LACTOSE (THREE FORMS), AND MALTOSE (ANHYDROUS AND CRYSTALLISED).

(For Lactose Figures see pages 58 to 61.)—*Continued.*

(Expressed in milligrams.)

Cuprous oxide (Cu₂O)	Copper (Cu)	Dextrose (d-glucose)	Invert sugar	Invert sugar and saccharose		Maltose		Cuprous oxide (Cu₂O)
				0.4 grm. total sugar	2 grm. total sugar	$C_{12}H_{22}O_{11}$	$C_{12}H_{22}O_{11}+H_2O$	
175	155.5	77.4	80.1	78.4	72.2	136.7	143.9	175
176	156.3	77.8	80.6	78.8	72.7	137.5	144.7	176
177	157.2	78.3	81.0	79.3	73.2	138.3	145.5	177
178	158.1	78.8	81.5	79.8	73.7	139.1	146.4	178
179	159.0	79.2	82.0	80.3	74.2	139.8	147.2	179
180	159.9	79.7	82.5	80.8	74.6	140.6	148.0	180
181	160.8	80.1	82.9	81.3	75.1	141.4	148.9	181
182	161.7	80.6	83.4	81.7	75.6	142.2	149.7	182
183	162.6	81.1	83.9	82.2	76.1	143.0	150.5	183
184	163.4	81.5	84.4	82.7	76.6	143.8	151.4	184
185	164.3	82.0	84.9	83.2	77.1	144.6	152.2	185
186	165.2	82.5	85.3	83.7	77.6	145.4	153.0	186
187	166.1	82.9	85.8	84.2	78.0	146.2	153.9	187
188	167.0	83.4	86.3	84.6	78.5	147.0	154.7	188
189	167.9	83.9	86.8	85.1	79.0	147.8	155.5	189
190	168.8	84.3	87.2	85.6	79.5	148.6	156.4	190
191	169.7	84.8	87.7	86.1	80.0	149.3	157.2	191
192	170.5	85.3	88.2	86.6	80.5	150.1	158.0	192
193	171.4	85.7	88.7	87.1	81.0	150.9	158.9	193
194	172.3	86.2	89.2	87.6	81.4	151.7	159.7	194
195	173.2	86.7	89.6	88.0	81.9	152.5	160.5	195
196	174.1	87.1	90.1	88.5	82.4	153.3	161.4	196
197	175.0	87.6	90.6	89.0	82.9	154.1	162.2	197
198	175.9	88.1	91.1	89.5	83.4	154.9	163.0	198
199	176.8	88.5	91.6	90.0	83.9	155.7	163.9	199
200	177.7	89.0	92.0	90.5	84.4	156.5	164.7	200
201	178.5	89.5	92.5	91.0	84.8	157.3	165.5	201
202	179.4	89.9	93.0	91.4	85.3	158.1	166.4	202
203	180.3	90.4	93.5	91.9	85.8	158.8	167.2	203
204	181.2	90.9	94.0	92.4	86.3	159.6	168.0	204
205	182.1	91.4	94.5	92.9	86.8	160.4	168.9	205
206	183.0	91.8	94.9	93.4	87.3	161.2	169.7	206
207	183.9	92.3	95.4	93.9	87.8	162.0	170.5	207
208	184.8	92.8	95.9	94.4	88.3	162.8	171.4	208
209	185.6	93.2	96.4	94.9	88.8	163.6	172.2	209
210	186.5	93.7	96.9	95.4	89.2	164.4	173.0	210
211	187.4	94.2	97.4	95.8	89.7	165.2	173.8	211
212	188.3	94.6	97.8	96.3	90.2	166.0	174.7	212
213	189.2	95.1	98.3	96.8	90.7	166.8	175.5	213
214	190.1	95.6	98.8	97.3	91.2	167.5	176.4	214
215	191.0	96.1	99.3	97.8	91.7	168.3	177.2	215
216	191.9	96.5	99.8	98.3	92.2	169.1	178.0	216
217	192.8	97.0	100.3	98.8	92.7	169.9	178.9	217
218	193.6	97.5	100.8	99.3	93.2	170.7	179.7	218
219	194.5	98.0	101.2	99.8	93.7	171.5	180.5	219
220	195.4	98.4	101.7	100.3	94.2	172.3	181.4	220
221	196.3	98.9	102.2	100.8	94.7	173.1	182.2	221
222	197.2	99.4	102.7	101.2	95.1	173.9	183.0	222
223	198.1	99.9	103.2	101.7	95.6	174.7	183.9	223
224	199.0	100.3	103.7	102.2	96.1	175.5	184.7	224
225	199.9	100.8	104.2	102.7	96.6	176.2	185.5	225
226	200.7	101.3	104.6	103.2	97.1	177.0	186.4	226
227	201.6	101.8	105.1	103.7	97.6	177.8	187.2	227
228	202.5	102.2	105.6	104.2	98.1	178.6	188.0	228
229	203.4	102.7	106.1	104.7	98.6	179.4	188.8	229

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR IN THE PRESENCE OF SACCHAROSE (0.4 GRM. AND 2 GRM. TOTAL SUGAR), LACTOSE (THREE FORMS), AND MALTOSE (ANHYDROUS AND CRYSTALLISED).

(For Lactose Figures see pages 58 to 61.)—*Continued.*

(Expressed in milligrams.)

Cuprous oxide (Cu₂O)	Copper (Cu)	Dextrose (d-glucose)	Invert sugar	Invert sugar and saccharose 0.4 grm. total sugar	2 grm. total sugar	Maltose C₁₂H₂₂O₁₁	C₁₂H₂₂O₁₁ + H₂O	Cuprous oxide (Cu₂O)
230	204.3	103.2	106.6	105.2	99.1	180.2	189.7	230
231	205.2	103.7	107.1	105.7	99.6	181.0	190.5	231
232	206.1	104.1	107.6	106.2	100.1	181.8	191.3	232
233	207.0	104.6	108.1	106.7	100.6	182.6	192.2	233
234	207.9	105.1	108.6	107.2	101.1	183.4	193.0	234
235	208.7	105.6	109.1	107.7	101.6	184.2	193.8	235
236	209.6	106.0	109.5	108.2	102.1	184.9	194.7	236
237	210.5	106.5	110.0	108.7	102.6	185.7	195.5	237
238	211.4	107.0	110.5	109.2	103.1	186.5	196.3	238
239	212.3	107.5	111.0	109.6	103.5	187.3	197.2	239
240	213.2	108.0	111.5	110.1	104.0	188.1	198.0	240
241	214.1	108.4	112.0	110.6	104.5	188.9	198.8	241
242	215.0	108.9	112.5	111.1	105.0	189.7	199.7	242
243	215.8	109.4	113.0	111.6	105.5	190.5	200.5	243
244	216.7	109.9	113.5	112.1	106.0	191.3	201.3	244
245	217.6	110.4	114.0	112.6	106.5	192.1	202.2	245
246	218.5	110.8	114.5	113.1	107.0	192.9	203.0	246
247	219.4	111.3	115.0	113.6	107.5	193.6	203.8	247
248	220.3	111.8	115.4	114.1	108.0	194.4	204.7	248
249	221.2	112.3	115.9	114.6	108.5	195.2	205.5	249
250	222.1	112.8	116.4	115.1	109.0	196.0	206.3	250
251	223.0	113.2	116.9	115.6	109.5	196.8	207.2	251
252	223.8	113.7	117.4	116.1	110.0	197.6	208.0	252
253	224.7	114.2	117.9	116.6	110.5	198.4	208.8	253
254	225.6	114.7	118.4	117.1	111.0	199.2	209.7	254
255	226.5	115.2	118.9	117.6	111.5	200.0	210.5	255
256	227.4	115.7	119.4	118.1	112.0	200.8	211.3	256
257	228.3	116.1	119.9	118.6	112.5	201.6	212.2	257
258	229.2	116.6	120.4	119.1	113.0	202.3	213.0	258
259	230.1	117.1	120.9	119.6	113.5	203.1	213.8	259
260	231.0	117.6	121.4	120.1	114.0	203.9	214.7	260
261	231.8	118.1	121.9	120.6	114.5	204.7	215.5	261
262	232.7	118.6	122.4	121.1	115.0	205.5	216.3	262
263	233.6	119.0	122.9	121.6	115.5	206.3	217.2	263
264	234.5	119.5	123.4	122.1	116.0	207.1	218.0	264
265	235.4	120.0	123.9	122.6	116.5	207.9	218.8	265
266	236.3	120.5	124.4	123.1	117.0	208.7	219.7	266
267	237.2	121.0	124.9	123.6	117.5	209.5	220.5	267
268	238.1	121.5	125.4	124.1	118.0	210.3	221.3	268
269	238.9	122.0	125.9	124.6	118.5	211.0	222.1	269
270	239.8	122.5	126.4	125.1	119.0	211.8	223.0	270
271	240.7	122.9	126.9	125.6	119.5	212.6	223.8	271
272	241.6	123.4	127.4	126.2	120.0	213.4	224.6	272
273	242.5	123.9	127.9	126.7	120.6	214.2	225.5	273
274	243.4	124.4	128.4	127.2	121.1	215.0	226.3	274
275	244.3	124.9	128.9	127.7	121.6	215.8	227.1	275
276	245.2	125.4	129.4	128.2	122.1	216.6	228.0	276
277	246.1	125.9	129.9	128.7	122.6	217.4	228.8	277
278	246.9	126.4	130.4	129.2	123.1	218.2	229.6	278
279	247.8	126.9	130.9	129.7	123.6	218.9	230.5	279
280	248.7	127.3	131.4	130.2	124.1	219.7	231.3	280
281	249.6	127.8	131.9	130.7	124.6	220.5	232.1	281
282	250.5	128.3	132.4	131.2	125.1	221.3	233.0	282
283	251.4	128.8	132.9	131.7	125.6	222.1	233.8	283
284	252.3	129.3	133.4	132.2	126.1	222.9	234.6	284

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR IN THE PRESENCE OF SACCHAROSE (0.4 GRM. AND 2 GRM. TOTAL SUGAR), LACTOSE (THREE FORMS), AND MALTOSE (ANHYDROUS AND CRYSTALLISED).

(For Lactose Figures see pages 58 to 61.)—*Continued.*

(Expressed in milligrams.)

Cuprous oxide (Cu₂O)	Copper (Cu)	Dextrose (d-glucose)	Invert sugar	Invert sugar and saccharose		Maltose		Cuprous oxide (Cu₂O)
				0.4 grm. total sugar	2 grm. total sugar	$C_{12}H_{22}O_{11}$	$C_{12}H_{22}O_{11}$ + H_2O	
285	253.2	129.8	133.9	132.7	126.6	223.7	235.5	285
286	254.0	130.3	134.4	133.2	127.1	224.5	236.3	286
287	254.9	130.8	134.9	133.7	127.6	225.3	237.1	287
288	255.8	131.3	135.4	134.3	128.1	226.1	238.0	288
289	256.7	131.8	135.9	134.8	128.6	226.9	238.8	289
290	257.6	132.3	136.4	135.3	129.2	227.6	239.6	290
291	258.5	132.7	136.9	135.8	129.7	228.4	240.5	291
292	259.4	133.2	137.4	136.3	130.2	229.2	241.3	292
293	260.3	133.7	137.9	136.8	130.7	230.0	242.1	293
294	261.2	134.2	138.4	137.3	131.2	230.8	242.9	294
295	262.0	134.7	138.9	137.8	131.7	231.6	243.8	295
296	262.9	135.2	139.4	138.3	132.2	232.4	244.6	296
297	263.8	135.7	140.0	138.8	132.7	233.2	245.4	297
298	264.7	136.2	140.5	139.4	133.2	234.0	246.3	298
299	265.6	136.7	141.0	139.9	133.7	234.8	247.1	299
300	266.5	137.2	141.5	140.4	134.2	235.5	247.9	300
301	267.4	137.7	142.0	140.9	134.8	236.3	248.8	301
302	268.3	138.2	142.5	141.4	135.3	237.1	249.6	302
303	269.1	138.7	143.0	141.9	135.8	237.9	250.4	303
304	270.0	139.2	143.5	142.4	136.3	238.7	251.3	304
305	270.9	139.7	144.0	142.9	136.8	239.5	252.1	305
306	271.8	140.2	144.5	143.4	137.3	240.3	252.9	306
307	272.7	140.7	145.0	144.0	137.8	241.1	253.8	307
308	273.6	141.2	145.5	144.5	138.3	241.9	254.6	308
309	274.5	141.7	146.1	145.0	138.8	242.7	255.4	309
310	275.4	142.2	146.6	145.5	139.4	243.5	256.3	310
311	276.3	142.7	147.1	146.0	139.9	244.2	257.1	311
312	277.1	143.2	147.6	146.5	140.4	245.0	257.9	312
313	278.0	143.7	148.1	147.0	140.9	245.8	258.8	313
314	278.9	144.2	148.6	147.6	141.4	246.6	259.6	314
315	279.8	144.7	149.1	148.1	141.9	247.4	260.4	315
316	280.7	145.2	149.6	148.6	142.4	248.2	261.2	316
317	281.6	145.7	150.1	149.1	143.0	249.0	262.1	317
318	282.5	146.2	150.7	149.6	143.5	249.8	262.9	318
319	283.4	146.7	151.2	150.1	144.0	250.6	263.7	319
320	284.2	147.2	151.7	150.7	144.5	251.3	264.6	320
321	285.1	147.7	152.2	151.2	145.0	252.1	265.4	321
322	286.0	148.2	152.7	151.7	145.5	252.9	266.2	322
323	286.9	148.7	153.2	152.2	146.0	253.7	267.1	323
324	287.8	149.2	153.7	152.7	146.6	254.5	267.9	324
325	288.7	149.7	154.3	153.2	147.1	255.3	268.7	325
326	289.6	150.2	154.8	153.8	147.6	256.1	269.6	326
327	290.5	150.7	155.3	154.3	148.1	256.9	270.4	327
328	291.4	151.2	155.8	154.8	148.6	257.7	271.2	328
329	292.2	151.7	156.3	155.3	149.1	258.5	272.1	329
330	293.1	152.2	156.8	155.8	149.7	259.3	272.9	330
331	294.0	152.7	157.3	156.4	150.2	260.0	273.7	331
332	294.9	153.2	157.9	156.9	150.7	260.8	274.6	332
333	295.8	153.7	158.4	157.4	151.2	261.6	275.4	333
334	296.7	154.2	158.9	157.9	151.7	262.4	276.2	334
335	297.6	154.7	159.4	158.4	152.3	263.2	277.0	335
336	298.5	155.2	159.9	159.0	152.8	264.0	277.9	336
337	299.3	155.8	160.5	159.5	153.3	264.8	278.7	337
338	300.2	156.3	161.0	160.0	153.8	265.6	279.5	338
339	301.1	156.8	161.5	160.5	154.3	266.4	280.4	339

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR IN THE PRESENCE OF SACCHAROSE (0.4 GRM. AND 2 GRM. TOTAL SUGAR), LACTOSE (THREE FORMS), AND MALTOSE (ANHYDROUS AND CRYSTALLISED).

(For Lactose Figures see pages 58 to 61.)—*Continued.*

(Expressed in milligrams.)

Cuprous oxide (Cu₂O)	Copper (Cu)	Dextrose (d-glucose)	Invert sugar	Invert sugar and saccharose		Maltose		Cuprous oxide (Cu₂O)
				0.4 grm. total sugar	2 grm. total sugar	$C_{12}H_{22}O_{11}$	$C_{12}H_{22}O_{11}$ + H_2O	
340	302.0	157.3	162.0	161.0	154.8	267.1	281.2	340
341	302.9	157.8	162.5	161.6	155.4	267.9	282.0	341
342	303.8	158.3	163.1	162.1	155.9	268.7	282.9	342
343	304.7	158.8	163.6	162.6	156.4	269.5	283.7	343
344	305.6	159.3	164.1	163.1	156.9	270.3	284.5	344
345	306.5	159.8	164.6	163.7	157.5	271.1	285.4	345
346	307.3	160.3	165.1	164.2	158.0	271.9	286.2	346
347	308.2	160.8	165.7	164.7	158.5	272.7	287.0	347
348	309.1	161.4	166.2	165.2	159.0	273.5	287.9	348
349	310.0	161.9	166.7	165.7	159.5	274.3	288.7	349
350	310.9	162.4	167.2	166.3	160.1	275.0	289.5	350
351	311.8	162.9	167.7	166.8	160.6	275.8	290.4	351
352	312.7	163.4	168.3	167.3	161.1	276.6	291.2	352
353	313.6	163.9	168.8	167.8	161.6	277.4	292.0	353
354	314.4	164.4	169.3	168.4	162.2	278.2	292.8	354
355	315.3	164.9	169.8	168.9	162.7	279.0	293.7	355
356	316.2	165.4	170.4	169.4	163.2	279.8	294.5	356
357	317.1	166.0	170.9	170.0	163.7	280.6	295.3	357
358	318.0	166.5	171.4	170.5	164.3	281.4	296.2	358
359	318.9	167.0	171.9	171.0	164.8	282.2	297.0	359
360	319.8	167.5	172.5	171.5	165.3	282.9	297.8	360
361	320.7	168.0	173.0	172.1	165.8	283.7	298.7	361
362	321.6	168.5	173.5	172.6	166.4	284.5	299.5	362
363	322.4	169.0	174.0	173.1	166.9	285.3	300.3	363
364	323.3	169.6	174.6	173.7	167.4	286.1	301.2	364
365	324.2	170.1	175.1	174.2	167.9	286.9	302.0	365
366	325.1	170.6	175.6	174.7	168.5	287.7	302.8	366
367	326.0	171.1	176.1	175.2	169.0	288.5	303.6	367
368	326.9	171.6	176.7	175.8	169.5	289.3	304.5	368
369	327.8	172.1	177.2	176.3	170.0	290.0	305.3	369
370	328.7	172.7	177.7	176.8	170.6	290.8	306.1	370
371	329.5	173.2	178.3	177.4	171.1	291.6	307.0	371
372	330.4	173.7	178.8	177.9	171.6	292.4	307.8	372
373	331.3	174.2	179.3	178.4	172.2	293.2	308.6	373
374	332.2	174.7	179.8	179.0	172.7	294.0	309.5	374
375	333.1	175.3	180.4	179.5	173.2	294.8	310.3	375
376	334.0	175.8	180.9	180.0	173.7	295.6	311.1	376
377	334.9	176.3	181.4	180.6	174.3	296.4	312.0	377
378	335.8	176.8	182.0	181.1	174.8	297.2	312.8	378
379	336.7	177.3	182.5	181.6	175.3	297.9	313.6	379
380	337.5	177.9	183.0	182.1	175.9	298.7	314.5	380
381	338.4	178.4	183.6	182.7	176.4	299.5	315.3	381
382	339.3	178.9	184.1	183.2	176.9	300.3	316.1	382
383	340.2	179.4	184.6	183.8	177.5	301.1	316.9	383
384	341.1	180.0	185.2	184.3	178.0	301.9	317.8	384
385	342.0	180.5	185.7	184.8	178.5	302.7	318.6	385
386	342.9	181.0	186.2	185.4	179.1	303.5	319.4	386
387	343.8	181.5	186.8	185.9	179.6	304.2	320.3	387
388	344.6	182.0	187.3	186.4	180.1	305.0	321.1	388
389	345.5	182.6	187.8	187.0	180.6	305.8	321.9	389
390	346.4	183.1	188.4	187.5	181.2	306.6	322.8	390
391	347.3	183.6	188.9	188.0	181.7	307.4	323.6	391
392	348.2	184.1	189.4	188.6	182.3	308.2	324.4	392
393	349.1	184.7	190.0	189.1	182.8	309.0	325.2	393
394	350.0	185.2	190.5	189.7	183.3	309.8	326.1	394

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR IN THE PRESENCE OF SACCHAROSE (0.4 GRM. AND 2 GRM. TOTAL SUGAR), LACTOSE (THREE FORMS), AND MALTOSE (ANHYDROUS AND CRYSTALLISED).

(Expressed in milligrams.)

Cuprous oxide (Cu₂O)	Copper (Cu)	Dextrose (d-glucose)	Invert sugar	Invert sugar and saccharose		Maltose		Cuprous oxide (Cu₂O)
				0.4 grm. total sugar	2 grm. total sugar	C₁₂H₂₂O₁₁	C₁₂H₂₂O₁₁ + H₂O	
395	350.9	185.7	191.0	190.2	183.9	310.6	326.9	395
396	351.8	186.2	191.6	190.7	184.4	311.4	327.7	396
397	352.6	186.8	192.1	191.3	184.9	312.1	328.6	397
398	353.5	187.3	192.7	191.8	185.5	312.9	329.4	398
399	354.4	187.8	193.2	192.3	186.0	313.7	330.2	399
400	355.3	188.4	193.7	192.9	186.5	314.5	331.1	400
401	356.2	188.9	194.3	193.4	187.1	315.3	331.9	401
402	357.1	189.4	194.8	194.0	187.6	316.1	332.7	402
403	358.0	189.9	195.4	194.5	188.1	316.9	333.6	403
404	358.9	190.5	195.9	195.0	188.7	317.7	334.4	404
405	359.7	191.0	196.4	195.6	189.2	318.5	335.2	405
406	360.6	191.5	197.0	196.1	189.8	319.2	336.0	406
407	361.5	192.1	197.5	196.7	190.3	320.0	336.9	407
408	362.4	192.6	198.1	197.2	190.8	320.8	337.7	408
409	363.3	193.1	198.6	197.7	191.4	321.6	338.5	409
410	364.2	193.7	199.1	198.3	191.9	322.4	339.4	410
411	365.1	194.2	199.7	198.8	192.5	323.2	340.2	411
412	366.0	194.7	200.2	199.4	193.0	324.0	341.0	412
413	366.9	195.2	200.8	199.9	193.5	324.8	341.9	413
414	367.7	195.8	201.3	200.5	194.1	325.6	342.7	414
415	368.6	196.3	201.8	201.0	194.6	326.3	343.5	415
416	369.5	196.8	202.4	201.6	195.2	327.1	344.4	416
417	370.4	197.4	202.9	202.1	195.7	327.9	345.2	417
418	371.3	197.9	203.5	202.6	196.2	328.7	346.0	418
419	372.2	198.4	204.0	203.2	196.8	329.5	346.8	419
420	373.1	199.0	204.6	203.7	197.3	330.3	347.7	420
421	374.0	199.5	205.1	204.3	197.9	331.1	348.5	421
422	374.8	200.1	205.7	204.8	198.4	331.9	349.3	422
423	375.7	200.6	206.2	205.4	198.9	332.7	350.2	423
424	376.6	201.1	206.7	205.9	199.5	333.4	351.0	424
425	377.5	201.7	207.3	206.5	200.0	334.2	351.8	425
426	378.4	202.2	207.8	207.0	200.6	335.0	352.7	426
427	379.3	202.8	208.4	207.6	201.1	335.8	353.5	427
428	380.2	203.3	208.9	208.1	201.7	336.6	354.3	428
429	381.1	203.8	209.5	208.7	202.2	337.4	355.1	429
430	382.0	204.4	210.0	209.2	202.7	338.2	356.0	430
431	382.8	204.9	210.6	209.8	203.3	339.0	356.8	431
432	383.7	205.5	211.1	210.3	203.8	339.7	357.6	432
433	384.6	206.0	211.7	210.9	204.4	340.5	358.5	433
434	385.5	206.5	212.2	211.4	204.9	341.3	359.3	434
435	386.4	207.1	212.8	212.0	205.5	342.1	360.1	435
436	387.3	207.6	213.3	212.5	206.0	342.9	361.0	436
437	388.2	208.2	213.9	213.1	206.6	343.7	361.8	437
438	389.1	208.7	214.4	213.6	207.1	344.5	362.6	438
439	390.0	209.2	215.0	214.2	207.7	345.3	363.4	439
440	390.8	209.8	215.5	214.7	208.2	346.1	364.3	440
441	391.7	210.3	216.1	215.3	208.8	346.8	365.1	441
442	392.6	210.9	216.6	215.8	209.3	347.6	365.9	442
443	393.5	211.4	217.2	216.4	209.9	348.4	366.8	443
444	394.4	212.0	217.8	216.9	210.4	349.2	367.6	444
445	395.3	212.5	218.3	217.5	211.0	350.0	368.4	445
446	396.2	213.1	218.9	218.0	211.5	350.8	369.3	446
447	397.1	213.6	219.4	218.6	212.1	351.6	370.1	447
448	397.9	214.1	220.0	219.1	212.6	352.4	370.9	448
449	398.8	214.7	220.5	219.7	213.2	353.2	371.7	449

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR IN THE PRESENCE OF SACCHAROSE (0.4 GRM. AND 2 GRM. TOTAL SUGAR), LACTOSE (THREE FORMS), AND MALTOSE (ANHYDROUS AND CRYSTALLISED).

(For Lactose Figures see pages 58 to 61.)—*Continued.*

(Expressed in milligrams.)

Cuprous oxide (Cu₂O)	Copper (Cu)	Dextrose (d-glucose)	Invert sugar	Invert sugar and saccharose		Maltose		Cuprous oxide (Cu₂O)
				0.4 grm. total sugar	2 grm. total sugar	$C_{12}H_{22}O_{11}$	$C_{12}H_{22}O_{11} + H_2O$	
450	399.7	215.2	221.1	220.2	213.7	353.9	372.6	450
451	400.6	215.8	221.6	220.8	214.3	354.7	373.4	451
452	401.5	216.3	222.2	221.4	214.8	355.5	374.2	452
453	402.4	216.9	222.8	221.9	215.4	356.3	375.1	453
454	403.3	217.4	223.3	222.5	215.9	357.1	375.9	454
455	404.2	218.0	223.9	223.0	216.5	357.9	376.7	455
456	405.1	218.5	224.4	223.6	217.0	358.7	377.6	456
457	405.9	219.1	225.0	224.1	217.6	359.5	378.4	457
458	406.8	219.6	225.5	224.7	218.1	360.3	379.2	458
459	407.7	220.2	226.1	225.3	218.7	361.0	380.0	459
460	408.6	220.7	226.7	225.8	219.2	361.8	380.9	460
461	409.5	221.3	227.2	226.4	219.8	362.6	381.7	461
462	410.4	221.8	227.8	226.9	220.3	363.4	382.5	462
463	411.3	222.4	228.3	227.5	220.9	364.2	383.4	463
464	412.2	222.9	228.9	228.1	221.4	365.0	384.2	464
465	413.0	223.5	229.5	228.6	222.0	365.8	385.0	465
466	413.9	224.0	230.0	229.2	222.5	366.6	385.9	466
467	414.8	224.6	230.6	229.7	223.1	367.3	386.7	467
468	415.7	225.1	231.2	230.3	223.7	368.1	387.5	468
469	416.6	225.7	231.7	230.9	224.2	368.9	388.3	469
470	417.5	226.2	232.3	231.4	224.8	369.7	389.2	470
471	418.4	226.8	232.8	232.0	225.3	370.5	390.0	471
472	419.3	227.4	233.4	232.5	225.9	371.3	390.8	472
473	420.2	227.9	234.0	233.1	226.4	372.1	391.7	473
474	421.0	228.5	234.5	233.7	227.0	372.9	392.5	474
475	421.9	229.0	235.1	234.2	227.6	373.7	393.3	475
476	422.8	229.6	235.7	234.8	228.1	374.4	394.2	476
477	423.7	230.1	236.2	235.4	228.7	375.2	395.0	477
478	424.6	230.7	236.8	235.9	229.2	376.0	395.8	478
479	425.5	231.3	237.4	236.5	229.8	376.8	396.6	479
480	426.4	231.8	237.9	237.1	230.3	377.6	397.5	480
481	427.3	232.4	238.5	237.6	230.9	378.4	398.3	481
482	428.1	232.9	239.1	238.2	231.5	379.2	399.1	482
483	429.0	233.5	239.6	238.8	232.0	380.0	400.0	483
484	429.9	234.1	240.2	239.3	232.6	380.7	400.8	484
485	430.8	234.6	240.8	239.9	233.2	381.5	401.6	485
486	431.7	235.2	241.4	240.5	233.7	382.3	402.4	486
487	432.6	235.7	241.9	241.0	234.3	383.1	403.3	487
488	433.5	236.3	242.5	241.6	234.8	383.9	404.1	488
489	434.4	236.9	243.1	242.2	235.4	384.7	404.9	489
490	435.3	237.4	243.6	242.7	236.0	385.5	405.8	490

Remarks.—It will be noticed that in this method it is recommended that the precipitate obtained should be weighed as cuprous oxide. This is undesirable for the reasons given on page 23, unless comparatively pure sugar solutions are being worked with. With the solutions obtained from ordinary plant products and from conversions by means of enzymes, etc., considerable error is undoubtedly incurred unless the copper is weighed as cupric oxide. The short time of heating is too, likely to cause error.

The writer can express no opinion as to the degree of accuracy of these tables but it may be pointed out, that it is extremely difficult to obtain pure maltose; all the so-called "pure" maltose on the market (Kahlbaum's, etc.) is indeed far from pure and in some cases contains 10 to 15% of dextrin. Such material can only be purified by repeated and laborious crystallisations (3 to 4 times) from 80% alcohol. The values given for maltose by Brown Morris and Millar (gravimetric) as well as those given by Ling and Jones (*infra*) (volumetric) can be relied upon.

Volumetric Methods.—Reasons are given on page 23 for considering that the method and tables due to Bertrand are not satisfactory for accurate work. On the other hand the volumetric method suggested by Ling (Vol. I, page 322[1]) is not only extremely rapid but is probably the most accurate volumetric method which has yet been suggested. The writer has thoroughly tested the tables given by Ling and Jones, using very carefully purified specimens of the different sugars, and can confirm the view generally held that this method is accurate to at least 1 part in 300 or 0.3%.

The following are the tables referred to. They refer to quantities of sugar solutions used to reduce 10 c.c. of Fehling solution, prepared as follows:

Solution No. 1.—Crystallised copper sulphate (69.2 grm.) is dissolved in water and the solution made up to 1 litre.

Solution No. 2.—Crystallised Rochelle salt (346 grm.) is dissolved in hot water and mixed with sodium hydroxide also dissolved in water (142 grm.). After cooling the mixed solutions are made up to 1 litre.

Equal volumes of these two solutions are accurately measured out at 15.5° (or any other standard temperature adopted) and this mixture constitutes the reagent. It is standardized as follows: Pure saccharose[3] (0.95 grm.) is dissolved in water (150 c.c.), and the solution boiled with 30 c.c. of $N/2$ hydrochloric acid exactly 1 minute; the solution is cooled, neutralised by adding $N/2$ sodium hydroxide (30 c.c.) and then diluted to 500 c.c. This solution, which contains 0.2 grm. of invert sugar per 100 c.c. is titrated against 10 c.c. portions of the copper reagent (see Allen, Vol. I, page 322).

The manner of using the table is best explained by an illustration. Suppose a solution of pure lævulose is being examined and that 25 c.c. of it are required to reduce 10 c.c. of Fehling's solution. Opposite 25 in the first column is found 0.2138 in column L; the percentage of lævulose in the solution titrated is thus given direct. If 25.2 c.c. or other quantity not a whole number is required to reduce 10 c.c. of Fehling's solution, the percentage of lævulose can easily be found by interpolation between the numbers given in column L.

The table gives the number of c.c. of Fehling's solution equivalent to 1 grm. of the particular sugar at each concentration. The numbers, given in columns D', L', I' and M' are used for another purpose—namely

[1] In Vol. I of Allen, page 322, line 6 from bottom, "2 seconds" should be 10 seconds.
[2] *Analyst*, 33, 160.
[3] See page 43.

TABLE III.—REDUCING POWER OF DIFFERENT SUGARS ACCORDING TO LING AND JONES.

Volume of solution required by 10 c.c. Fehling's solution	Dextrose		Lævulose		Invert sugar		Maltose	
	D Dextrose in 100 c.c. of solution	D' Fehling's solution equivalent to 1 grm. dextrose	L Lævulose in 100 c.c. of solution	L' Fehling's solution equivalent to 1 grm. lævulose	I Invert in 100 c.c. of solution	I' Fehling's solution equivalent to 1 grm. invert	M Maltose in 100 c.c. of solution.	M' Fehling's solution equivalent to 1 grm. maltose
C.c.	Gram.	C.c.	Gram.	C.c.	Gram.	C.c.	Gram.	C.c.
20	0.2427	206.0	....	....	....	....	....	....
21	0.2332	205.1	....	....	0.2412	197.5	0.3888	122.5
22	0.2226	204.2	0.2411	188.5	0.2311	196.8	0.3711	
23	0.2138	203.4	0.2312	188.0	0.2218	196.0	0.3550	
24	0.2056	202.6	0.2222	187.5	0.2132	195.5	0.3402	
25	0.1981	201.9	0.2138	187.1	0.2052	194.9	0.3266	
26	0.1911	201.3	0.2060	186.7	0.1980	194.3	0.3140	
27	0.1846	200.7	0.1988	186.3	0.1910	193.9	0.3023	
28	0.1784	200.1	0.1921	186.0	0.1846	193.4	0.2915	
29	0.1728	199.6	0.1857	185.6	0.1787	193.0	0.2815	
30	0.1675	199.1	0.1798	185.4	0.1731	192.5	0.2721	
31	0.1625	198.6	0.1743	185.1	0.1678	192.2	0.2633	A constant.
32	0.1577	198.2	0.1691	184.8	0.1629	191.8	0.2551	
33	0.1532	197.8	0.1642	184.6	0.1583	191.5	0.2474	
34	0.1490	197.4	0.1596	184.3	0.1539	191.2	0.2401	
35	0.1450	197.0	0.1552	184.1	0.1497	190.9	0.2332	
36	0.1412	196.7	0.1511	183.9	0.1458	190.6	0.2268	
37	0.1377	196.4	0.1472	183.6	0.1421	190.3	0.2206	
38	0.1343	196.0	0.1435	183.4	0.1385	190.1	0.2148	
39	0.1310	195.8	0.1399	183.3	0.1349	189.8	0.2093	
40	0.1279	195.5	0.1366	183.1	0.1319	189.6	0.2041	122.5
41	....	....	0.1334	182.9	0.1288	189.4	....	....
42	....	....	0.1298	182.8	0.1259	189.2	....	....
43	....	....	0.1274	182.6	....	....	....	....

the separate determination of two reducing sugars in a mixture by a modification of Morris' method[1] (see pages 9 and 10).

Estimation of Invert Sugar in Presence of Cane Sugar.—Ling and Rendle (*Analyst*, 1908, 33, 170) have determined the corrections to be applied in order to allow for the influence of the saccharose when present in mixtures of sugars. In the following table IV:

Column A gives the amount in grams of saccharose present in 100 c.c. of the sugar solution.

Column B gives the percentage of saccharose present expressed on the total sugars.

Column C gives the percentage of invert sugar present expressed on the total sugars.

Column D gives the number of c.c. of sugar solution required to reduce 10 c.c. of Fehling's solution.

Column E gives the percentage of invert sugar on the total sugars found by direct experiment.

Column F gives the differences between the values shown in columns C and E.

The influence of the saccharose is seen to be practically negligible until the proportion to the total sugars (saccharose + invert sugar) amounts to 30%, at which point the invert sugar is overestimated by 0.2%. The in-

[1] *J. Inst. Brewing,* 4, 162.

TABLE IV.—EACH SOLUTION CONTAINED IN ADDITION TO THE SAC-CHAROSE SHOWN UNDER COLUMN A, 0.2 GRM. OF INVERT SUGAR PER 100 C.C.

A	B	C	D	E	F
0.01	4.8	95.2	25.60	95.30	0.10
0.03	13.0	87.0	25.60	87.10	0.10
0.05	20.0	80.0	25.60	80.10	0.10
0.10	33.3	66.7	25.55	66.90	0.20
0.20	50.0	50.0	25.45	50.40	0.40
0.30	60.0	40.0	25.40	40.40	0.40
0.40	66.6	33.4	25.35	33.60	0.40
0.50	71.4	38.6	85.30	29.00	0.40
0.60	75.0	25.0	25.20	25.40	0.40
0.70	77.7	22.3	25.15	22.70	0.40
0.80	80.0	20.0	25.10	20.40	0.40
1.25	86.2	13.8	25.05	14.10	0.30
1.50	88.2	11.8	24.95	12.10	0.30
1.75	89.7	10.3	24.85	10.60	0.30
1.75	89.7	10.3	24.80	10.60	0.30
2.00	90.9	9.1	24.70	9.45	0.35
2.00	90.9	9.1	24.80	9.41	0.31
2.50	92.5	7.5	24.80	7.76	0.26
3.00	93.8	6.2	24.70	6.44	0.24
5.00	96.1	3.9	24.20	4.05	0.15
7.00	97.2	2.8	23.60	3.04	0.24
10.00	98.0	2.0	22.95	2.23	0.23
20.00	99.0	1.0	22.40	1.14	0.14
25.00	99.2	0.8	22.25	0.92	0.12
30.00	99.3	0.7	22.25	0.80	0.10

fluence of the saccharose increases progressively until the proportion of the latter expressed on the total sugars is 99.3%, beyond which point it was not determined. At this point the invert sugar is overestimated by about 15%. It must be remembered that the magnitudes representing the percentages of invert sugar decrease as the percentage of saccharose increases, and the correction to be applied (column F) is in concrete numbers greatest when the percentage of saccharose in the total sugars is between 50 and 80%. In the case of a mixture of equal parts of saccharose and invert sugar the latter would be returned, if no correction were made, as 50.4 instead of 50.0%, whilst in the case of a mixture of 99 parts of saccharose and 1 of invert sugar the latter would be returned as 1.14% instead of 1.0%. Ling generally deducts the values shown in column F from the values ascertained by direct titration of the mixture of sugars. For this purpose it is necessary to know the percentage of saccharose not calculated on the sample, but on the total sugars (saccharose + invert sugar) in the sample. This can be determined by the Clerget method or by the method of double titration before and after hydrolysis with hydrochloric acid, applying the formula $S = \dfrac{95 I' - I}{100}$ in which S is the approximate percentage of saccharose, I the apparent percentage of invert sugar, i.e., the value obtained by direct titration, and I' is the percentage of invert sugar obtained by titration after complete hydrolysis.

The approximate value given in column F is subtracted from the value of I and added to the value of S, the respective results giving the corrected percentages of invert sugar and of saccharose. In order to express these on the sample each of the values is multiplied by S+I/100. To be exact

the value added to the approximate percentage of saccharose, S, should be diminished by 5%; but the degree of accuracy of the method does not warrant this refinement, seeing that the corrections to be applied are comparatively small.

Low's Volumetric Method (Provisional A. O. A. C. Method).—In this method, the copper in the precipitate of cuprous oxide obtained by the action of a sugar solution on Fehling's solution is estimated iodometrically. The method prescribed by the A. O. A. C. is as follows (Bulletin 107, revised 1912, page 241):[1]

LOW'S VOLUMETRIC METHOD, MODIFIED.[2] PROVISIONAL.

(a) Standardisation of the Thiosulphate Solution.

Prepare a solution of sodium thiosulphate containing 19 grm. of pure crystals to 1,000 c.c. Weigh accurately about 0.2 grm. of pure copper foil and place in a flask of 250 c.c. capacity. Dissolve by warming with 5 c.c. of a mixture of equal volumes of strong nitric acid and water. Dilute to 50 c.c., boil to expel the red fumes, add 5 c.c. of strong bromine water, and boil until the bromine is thoroughly expelled. Remove from the heat and add a slight excess of strong ammonium hydroxide—7 c.c. is about the right amount. Again boil until the excess of ammonia is expelled, as shown by a change of colour of the liquid and a partial precipitation. Now add a slight excess of strong acetic acid (3 or 4 c.c. of 80% acid) and boil for a minute. Cool to room temperature and add 10 c.c. of a solution of pure potassium iodide containing 300 grm. of potassium iodide to 1,000 c.c. Titrate at once with the thiosulphate solution until the brown tinge has become weak, then add sufficient starch liquor to produce a marked blue colouration. Continue the titration cautiously until the colour due to free iodine has entirely vanished. The blue colour changes toward the end to a faint lilac. If at this point the thiosulphate be added drop by drop and a little time be allowed for complete reaction after each addition, there is no difficulty in determining the end point within a single drop. 1 c.c. of the thiosulphate solution will be found to correspond to about 0.005 grm. of copper.

(b) Determination of Copper.

After washing the precipitated cuprous oxide, cover the Gooch with a watch glass and dissolve the oxide by means of 5 c.c. of warm nitric acid (1:1) poured under the watch glass with a pipette. Catch the filtrate in a flask of 250 c.c. capacity, wash the watch glass and Gooch free of copper; 50

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[1] In a modification of the iodometric method suggested by Schoorl (*Zeit. angew. Chem.*, 1899, 633) the copper remaining in the Fehling's solution after treatment with the sugar solution is estimated and the difference between this value and that given by the Fehling's solution alone is a measure of the copper precipitate. This process obviates the necessity of filtering off the precipitate. Schoorl gives tables.

[2] *J. Amer. Chem. Soc.*, 1902, 24, 1082.

c.c. of water will be sufficient.   Boil to expel red fumes, add 5 c.c. of bromine water, boil off the bromine, and proceed exactly as in standardising the thiosulphate.

Estimation of Small Quantities of Reducing Sugars in Presence of a Large Quantity of Saccharose.

It is well known that when large quantities of cane sugar are present, the values obtained for the reducing sugars, present in relatively small proportion, are considerably increased; this is probably due to the fact that, under the ordinary conditions of working, when the action takes place at the boiling point, traces of the saccharose are hydrolysed and so increase the actual amount of invert sugar present. Pellet[1] has made a special study of this question and points out that the amount of reduction obtained on heating Fehling's solution with solutions of cane sugar varies with: (1) the time of ebullition; (2) the proportion of Fehling's solution relative to the sugar; (3) the quantity of cane sugar; (4) the proportion of alkaline hydroxide in the Fehling's solution.

The effect of cane sugar on Fehling's solution according to Pellet is obviated by carrying out the heating, not at 100° as is usual, but at 60–62°; at this temperature there is not a trace of cuprous oxide formed when 10 grm. of pure cane sugar is heated for 10 minutes with 20 c.c. of Fehling's solution. Pellet suggests, therefore, the following process of estimating small quantities of reducing sugar in presence of large quantities of saccharose.

Pellet's Method.—The Fehling's solution used is prepared as follows:
Solution A. 34.64 grm. of copper sulphate made up to 500 c.c.
Solution B.   Rochelle salt,   180 grm.
                    Sodium hydroxide (sticks),   60 grm.
                    Water to make to 500 c.c.
This solution contains a relatively small proportion of sodium hydroxide and it may be noted, is very nearly the same in composition as that adopted by Brown, Morris and Millar (page 25). 10 c.c. of the mixed solution corresponds with 0.050 grm. of invert sugar.

To estimate the quantity of reducing sugar in highly purified and half-purified cane and beet products, 25 grm. of this material are dissolved in 100 c.c. of water; 20 c.c. of this solution is mixed with 20 c.c. of the Fehling's solution and 10 c.c. of water and the mixture heated in a beaker flask (see page 26) during 10 minutes in a water-bath at 60–62°. 50 c.c. of cold water are then added and the cuprous oxide collected at once, thoroughly washed and ignited to cupric oxide. Pellet makes use of a filter paper to collect the precipitate, in which case a blank experiment has to be made, with the same volume of Fehling's solution, in order to ascertain how much copper is taken up by the filter paper itself under exactly similar conditions.

[1] *Bull. Assoc. Chim. Sucr.*, 1913, **31**, 182.

Pellet suggests using small filter papers having a diameter of about 3 cm. in conjunction with a special filtering arrangement devised for the purpose. With these small discs of filter paper the method can be used colourimetrically, the proportion of cuprous oxide being judged by the depth of colour on the disc of filter paper of known size; for this purpose a series of standards are prepared by means of known very small quantities of invert sugar. Under the conditions Pellet used, the weight of reducing sugar (invert sugar) is calculated by multiplying the weight of cupric oxide (CuO) by 0.453. But the value of the constant will vary slightly according to the dimensions of the vessel in which the mixture of sugar and Fehling's solution is heated, that is, according to the proportion of the free surface to the depth, so that each worker should ascertain for himself the value of this ratio for his own conditions, by using a dilute solution of invert sugar (prepared from pure cane sugar) of known concentration.

Pellet states that by means of this method the true proportions of reducing sugar can be determined in even the highest grades of crystallised cane sugar, which are generally returned as free from invert sugar as they give less than 50 mg. of copper under the Herzfeld conditions (Vol. I, page 329) the generally unsatisfactory character of this method when used to estimate small quantities of invert sugar in cane sugar is shown by the fact that samples of pure saccharose, absolutely free from reducing sugar according to Pellet's method, will give as much as 100 mg. of cupric oxide when 10 grm. of the material are heated for 3 minutes with Fehling's solution at the boil. On the other hand, Pellet states that his method of working at 60° enables one readily to discriminate between sugars which are practically free from invert sugar and those which contain 0.01 or 0.02%. The exact quantity of the invert sugar in the latter cases can easily be estimated.

Saccharose.

Estimation by Inversion.—In spite of the numerous researches which have been carried out during the past 20 years with the object of standardising the method of estimating cane sugar by inversion, it is now generally recognised that the Clerget-Herzfeld process is not entirely satisfactory in many cases. This is especially true of the estimation of saccharose in the mother liquors and molasses of sugar factories. In such cases, many possibilities of error arise. These have been recently summarised by H. Pellet in a monograph *Dosage du Sucre par Inversion* originally published in *La Sucrerie Indigène*, 1913, which forms a valuable survey of the whole question. The following is a brief summary of the principal sources of error in this method.

(1) It is necessary to make allowance for the change in the specific rotation of cane sugar caused by dilution. In the ordinary Clerget-Herzfeld formula the constant used, viz., 142.66 is correct only when the quan-

tity of cane sugar present is the half-normal weight, namely 13.0 grm.[1] In the case of products such as crude sugars and molasses, in which the actual sugar may be only 45%, it is necessary to use the constant which applies to the particular concentration of the saccharose actually present. This may be obtained from the following table, due to Herzfeld in 1888; or it can be determined directly by making a control observation with a solution of pure saccharose of approximately the same concentration as that present in the actual analysis. The latter method is really preferable because in this way any error in the instrument or in the graduation of vessels, etc., is allowed for.

TABLE V.
ALTERATION OF HERZFELD CONSTANT WITH CONCENTRATION OF SACCHAROSE.

Grm. saccharose per 100 c.c.	Constant in Herzfeld formula	Grm. saccharose per 100 c.c.	Constant in Herzfeld formula
1	141.85	11	142.52
2	141.91	12	142.59
3	141.98	13	142.66
4	142.05	14	142.73
5	142.12	15	142.79
6	142.18	16	142.86
7	142.25	17	142.93
8	142.32	18	143.00
9	142.39	19	143.07
10	142.46	20	143.14

(2) The principal source of error in estimating sugars in beet molasses or in vegetable extracts in general, is due to the presence of amic acids or acid amides (such as glutamine, glutamic acid, asparagine and aspartic acids) which have a decided specific rotatory power; the error arises in the ordinary inversion process owing to the fact that such substances have a very different specific rotation in aqueous solution, in presence of basic lead acetate and in presence of hydrochloric acid. Pellet gives data for the amides named above, which show that in some cases, for example that of glutamic acid, a solution which is strongly lævorotatory in presence of a slight excess of basic lead acetate becomes strongly dextrorotatory when the solution is made acid. Consequently, the difference between the direct reading, taken in presence of basic lead acetate, and that obtained in acid solution, after inversion by the Herzfeld process, does not in such cases give a true measure of the cane sugar present; the change of rotation found is less than that actually due to the sugar present, which is consequently returned at a lower figure than is actually correct. There is also an error due to the transformation of an amide such as glutamine or asparagine into an acid such as glutamic acid, by partial hydrolysis, this change being accompanied by a change of rotation.

[1] Stanek (*Zeit. Zuckerind. Böhm*, 1914, 38, 289), states that the constant 142.66, for this concentration, strictly holds good only when the inverted solution is polarised within 3 to 5 minutes; if the examination is delayed, as for instance, when the solution has to be decolourised, for 15 to 30 minutes the value 142.66 is too low, and the constant 143 should then be used.

Errors caused in this way by acid amides such as glutamine and aspara-gine (which are not eliminated by precipitation with basic lead acetate) are largely obviated by taking the *direct* polarisation reading in acid solution, according to Pellet's process (see below), using sulphurous acid instead of hydrochloric acid. This serves not only to remove the excess of lead by precipitation as sulphite, but at the same time clarifies and bleaches the solution so that it can easily be read in the polarimeter. The direct reading being taken in acid solution, is really comparable with the reading after inversion.

Pellet's New Method of Estimating Saccharose in Molasses.—100 grm. of the molasses are placed in a 500 c.c. flask together with water so as to make the volume equal to about 300 c.c.; after thoroughly mixing, basic lead acetate is gradually added until there is no further precipitate. This addition is made as carefully as possible so as to avoid having any considerable excess of the solution of lead. The solution, without filtering, is then diluted to 500 c.c., well shaken, and filtered. Take the *direct polarisation* of the filtrate (1).

Transfer 50 c.c. of the filtrate to a dry 100 c.c. measuring flask and make up to 100 c.c. with distilled water. If the measuring vessels, etc., are correct, the polarisation should be exactly half that formerly obtained. Another 50 c.c. of the filtrate are placed in a 100 c.c. flask and about 49 c.c. of sul-phurous acid solution of sp. gr. 1.040 to 1.045 added[1] and the solution is diluted to 100 c.c. It is then carefully shaken, and 1 grm. of specially pure decolourising carbon (which must be without action on the sugars present) is added and the solution again agitated; it is then filtered and the polari-sation observed, preferably in a 400 mm. tube. The result is multiplied by 0.9985, so as to take into account the volume of the precipitated lead sul-phite. This gives the *acid direct polarisation* (2).

50 c.c. of the filtrate from the lead precipitate are placed in a 100 c.c. flask (A) with 5 c.c. of concentrated hydrochloric acid (sp. gr. 1.118) + 25 c.c. of sulphurous acid, sp. gr. 1.040 − 1.045. A second quantity is prepared similarly in a flask (B) and in a dried flask which serves as a temperature control, 80 c.c. of water are placed; all these flasks are then placed in a boiling water-bath, until the temperature of the control is exactly 70°; this is the case after 2½ to 3 minutes. The three flasks are then transferred to a water-bath heated at 70°, and flask A is heated 7 minutes and flask B 10 minutes at 70°. The extra 3 minutes heating in flask B ensures that inversion is com-plete and gives a check on the result obtained in flask A.

After the inversion the solutions A and B are cooled and diluted to 100 c.c. 1 grm. of pure decolourising carbon is added to each, the solutions are shaken, filtered and the polarisation taken (in a 400 mm. tube) (3). The cane sugar is calculated from the acid polarisation before inversion and the polarisation

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[1] If one has sulphur-dioxide in siphon, it is sufficient to pass the gas through the 50 c.c. of solution for 2 to 3 minutes and then make up to 100 c.c. Ogilvie (*Int. Sugar J.*, 1912, 14, 624) showed that the original Pellet process gave a low figure for saccharose owing to an insufficiency of sulphur dioxide being used. With the modified process as given above, values are obtained which are identical with those obtained by invertase or by the Andrlik method.

after inversion. Instead of using the usual constant 142.66, the value in Table V, which corresponds with the approximate quantity of sugar present should be employed.

Use of Invertase in Estimating Saccharose.—The use of the selective enzyme *invertase* has certain advantages in estimating cane sugar in complex mixtures such as molasses or the mixtures of sugars that are obtained on extracting leaves or other plant tissues. The enzyme brings about hydrolysis of cane sugar quite rapidly and completely at the ordinary temperature or slightly above it (the optimum temperature is 38°) and is purely selective in its action; it does not hydrolyse maltose or similar glucosides, and therefore is more discriminative in its action than hydrochloric acid under Herzfeld conditions. Ogilvie[1] has applied it to the analysis of beet and cane molasses and Davis and Daish[2] make use of it in the form of autolysed yeast (Vol. I, p. 314)[3] in their scheme of analysis of plant extracts (p. 64) following in this the example of Brown and Morris (*Trans.*, 1893, **63**, 663). Ogilvie has shown that invertase gives practically the same results for molasses as Pellet's sulphurous acid method and both values are in close agreement with the values found by the Andrlik method[4] in which hydrochloric acid is used and urea is added to stop the invertive action of the acid on the cane sugar.

TABLE VI.—CANE MOLASSES.

	Cuban molasses		Egyptian molasses	Javan molasses	American syrup
	No. 1	No. 2			
Ash (sulphated).............................	6.77	7.34	10.92	10.97	6.05
Reducing sugars............................	18.71	18.56	11.70	21.98	26.55
1. Alkaline (basic lead acetate) polarisation...	32.20	31.40	39.50	34.30	39.70
2. Neutral direct polarisation...............	30.50	29.90	38.10	33.46	39.65
3. Acid (HCl+urea) polarisation (Andrlik)...	30.50	30.50	38.40	33.38	39.26
4. Acid (SO₂) direct polarisation (Pellet).....	30.60	30.50	38.30	33.30	39.10
5. Invertase inversion polarisation...........	-16.40	-15.50	-17.90	-14.08	-11.40
6. Acid inversion (Herzfeld) polarisation......	-16.30	-15.40	-18.02	-14.06	-12.28
7. % saccharose, using invertase............	35.6	34.40	42.4	36.1	38.7
8. % saccharose using acid as hydrolyst and the alkaline direct polarisation.	36.7	35.40	43.4	36.6	39.3
9. % Saccharose using acid as hydrolyst and the neutral direct polarisation.	35.4	34.2	42.4	35.9	39.2
10. % saccharose using acid as hydrolyst and (HCl+urea) direct polarisation (Andrlik).	35.4	34.7	42.6	35.9	38.9
11. Saccharose, using acid as hydrolyst and (SO₂) direct polarisation (Pellet).	35.5	34.7	42.5	35.8	38.8

[1] *Int. Sugar J.*, 1912, **14**, 89 and 624.

[2] *J. Agric. Sci.*, 1913, **5**, 437.

[3] Hudson and Paine (*J. Amer. Chem. Soc.*, 1914, **36**, 1566) have recently described a rather tedious method of preparing an invertase solution for cane sugar inversions, in which the solution from the autolysed yeast is clarified with lead acetate, the excess of lead removed with hydrogen sulphide and the liquid dialysed through a collodion sac in running water. This treatment is quite unnecessary to prevent the solution from undergoing change and also involves considerable loss of activity of the invertase preparation. A solution prepared in the following way can be kept 2 or 3 years without appreciably losing its activity. About ½ lb. of fresh brewer's yeast, after being thoroughly washed with water in a Buchner funnel to remove wort, is packed into a wide-necked bottle and, after adding about 50 c.c. of toluene is left to undergo autolysis for several days at about 38°; the yeast nearly completely liquefies, and the liquid, which is only very slightly coloured, is filtered from the solid residue. It is kept in a stoppered bottle in the dark. About 10 to 20 c.c. of toluene should be added, so as to keep a film of this liquid floating on the surface of the preparation and exclude air.

[4] *Zeit. Zuckind. Böhm*, 1906, **31**, 417.

TABLE VII.—BEET MOLASSES.

(Ogilvie, *J. Soc. Chem. Ind.*, 1911, 30, 62.)

	Sample 1	Sample 2	Sample 3	Sample 4
1. Direct alkaline (basic lead) polarisation..........	48.8	....	48.6	47.0
2. Direct neutral polarisation......................	49.2	52.0	49.0	47.6
3. Direct acid (Andrlik) polarisation...............	50.4	53.25	50.0	48.4
4. Direct acid (SO₂) polarisation (Pellet)...........	50.3	53.3	....	48.3
5. Invertase inversion polarisation.................	−14.6	−16.0	−15.0	−14.4
6. Acid inversion polarisation......................	−13.2	−14.6	−13.6	−13.8
7. Clerget value by invertase......................	48.5	51.7	48.6	47.1
8. Clerget value by acid, using alkaline direct polarisation.	47.0	....	47.1	46.1
9. Clerget value by acid, using neutral direct polarisation.	47.3	50.5	47.4	46.5
10. Clerget value by acid, using acid (Andrlik) direct polarisation.	48.2	51.4	48.2	47.1
11. Clerget value by acid, using acid (SO₂) direct polarisation (Pellet).	48.1	51.4	....	47.0

From these tables it is seen that with *beet* molasses the results obtained by the ordinary Herzfeld process using the alkaline direct polarisation are *low* as compared with the practically identical values obtained using invertase or the direct acid polarisation. In the case of *cane* molasses the reverse is true, the results obtained by the ordinary method being *high*. The difference is probably due to the different character of the disturbing factor in the two cases: in beet molasses it is the presence of amides and amino-acids and in cane molasses it is the reducing sugars.

According to Cross and Taggart[1] the retarding action of betaine and urea on the inversion of saccharose by hydrochloric acid is only slight at temperatures of 20 to 28° and therefore analytical methods based on this supposed retarding influence, such as the Andrlik process are not satisfactory. According to Pellet (*loc. cit.*) in the Andrlik method the polarisation of the acid solution should be completed in less than 7 minutes if change of the saccharose is to be avoided.

There is perhaps still some doubt whether the values obtained by the Pellet and Andrlik processes actually represent the true saccharose present; the agreement between the results obtained by the two methods may be fortuitous and it is worthy of note that the original Pellet method, in which a smaller proportion of sulphur dioxide was used, gave slightly different results and has been modified by increasing the amount of sulphur dioxide present. On the other hand, it is a confirmation of some value that the invertase method gives practically identical results (Ogilvie). It would, however, be interesting to ascertain whether the results obtained for cane sugar in such cases, by measuring the increase of reducing power by Fehling's solution (gravimetrically or volumetrically), correspond with those given by the change of rotation measured under the various conditions given above. Owing to the great influence of salts, acids and neutral substances such as betaine or urea on the specific rotatory power of the sugars and other optic-

[1] *Louisiana State University Agriculture Bulletin*, No. 135, December, 1912.

ally active substances present, a certain amount of doubt must be felt as to the strict reliability of methods which rest on the polarisation figures only, until it has been finally put beyond question by other methods that these are correct.

Inversion of Saccharose by Citric Acid.—In estimating saccharose in plant extracts it is inadvisable to invert with hydrochloric acid at 70° under Herzfeld conditions as if maltose is present a certain proportion undergoes hydrolysis to dextrose.[1] In such cases a weaker acid such as citric acid can be applied but error may arise unless special precautions are taken. Davis and Daish[2] show that in a series of experiments carried out on mangold leaf extracts from which tannins, amino-acids, etc., had been as far as possible removed by basic lead acetate in the usual way, the amount of cane sugar found by inverting with 2% citric acid was very small as compared with the amount found by means of invertase. It was ultimately discovered that the cause of the difference was the presence in solution of a relatively large proportion of sodium acetate, which almost entirely inhibits the invertive action of 2% citric acid. The sodium acetate was produced owing to the necessity, in the cases dealt with, of using relatively large quantities of basic lead acetate to remove the tannins, etc., present in the extracts analysed; on subsequently adding sodium carbonate to precipitate the slight excess of lead present in the filtered solution the whole of the acetic acid originally present in the basic acid acetate was converted into sodium acetate. It is an interesting fact that although sodium acetate, when present to the extent of about 1 to 2%, almost completely inhibits inversion of cane sugar by boiling 2% citric acid, it does not in the least interfere with the action of invertase (autolysed yeast); this shows the advantage of using invertase in many cases as a means of checking the results obtained by acid hydrolysis. The writer in estimating cane sugar in plant material invariably uses two methods (see page 65): (1) hydrolysis by boiling 10% citric acid; (2) hydrolysis during 24 hours at 38° by 1 c.c. of autolysed yeast (see footnote on page 46). The results mutually check each other. The method of carrying out the hydrolysis by 10% citric acid is as follows: 50 c.c. of the solution to be analysed (which has had the excess of lead removed by means of sodium carbonate) is treated with a few drops of concentrated sulphuric acid until it just shows the faintest indication of pink to methyl-orange. Solid citric acid is then added so as to make a 10% solution (5 grm. solid citric acid to 50 c.c. of solution) and the mixture raised to the boil; it is kept actively boiling over a small flame for 10 minutes, under a reflux condenser, and the solution is then cooled to the ordinary temperature and exactly neutralised (using phenolphthaleïn as indicator) by adding a 10 or 15% solution

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[1] It is generally stated that maltose is not hydrolysed at all by hydrochloric acid under Herzfeld conditions and Kluyver (*Biochemische Suikerbepalingen*, 1914, page 223) considered that Davis and Daish were in error in considering this statement to be incorrect; the writer has however since shown (Davis, *J. Agric. Sci.*, 1914, **6**, 413) that under Herzfeld conditions the hydrolysis of maltose is quite appreciable and sufficient to interfere with the accuracy of the cane sugar estimation.

[2] *J. Agric. Sci.*, 1913, **5**, 437.

of sodium hydroxide. It is diluted to 100 c.c. at 15° (or 20° according to the temperature at which the flask is graduated) and is then ready for the polarisation or for measuring the reducing power by means of Fehling's solution.

In all cases that the writer has yet met, 10% citric acid under the conditions given has effected complete hydrolysis of the cane sugar even when very large quantities of basic lead acetate have been employed in the preliminary purification of the solutions dealt with. Special experiments have shown that no maltose is hydrolysed under these conditions.

Influence of Basic Lead Acetate in Sugar Analysis.—It has been generally considered since the work of Gill,[1] Pellet,[2] Edson,[3] and Geerligs[4] that when basic lead acetate is used in purifying and decolorising solutions containing reducing sugars, a considerable proportion of the latter is thrown out of solution; this action is most marked in the case of lævulose and Pellet has stated that in some cases 23% of the lævulose present is precipitated at the ordinary temperature, whilst at 50° this sugar is totally removed. Whilst the experimental results recorded by the above authors are perfectly correct, the writer, from a series of experiments as yet unpublished, has reason to doubt the correctness of the view that lævulose is precipitated under the conditions named above. It has been found that, at least in dilute solutions, lævulose is not *precipitated* at all by basic lead acetate, even in presence of impurities such as sodium sulphate or organic acids, and that no loss occurs *unless the solution of the lævulose is left in contact with the basic lead acetate for some time.* If to the solution containing the lævulose, basic lead acetate is added in not too large excess (5 c.c. or 10 c.c.) and then the lead is precipitated immediately by sodium sulphate or sodium carbonate, practically 100% of the lævulose is recovered in the solution. On the other hand, if the lævulose is left with the basic lead acetate for various lengths of time, for example, 1 hour, 24 hours, 48 hours, and the lead is subsequently precipitated by the same reagents, different proportions of sugar are found to have disappeared; the amount apparently lost increases with the time that the solution has been standing in contact with the basic lead acetate. At the same time the lævulose solution progressively becomes more and more yellow in colour, without, however, any precipitation becoming visible. It would appear that, instead of the lævulose being precipitated in the form of an insoluble lead compound, as has been generally supposed to be the case, what happens is that the lævulose is either destroyed by the basic lead acetate (possibly by an oxidising action which occurs in the alkaline solution) or it is transformed into another carbohydrate with a different specific rotatory power and a smaller reducing power. Lobry de Bruyn and

[1] *J. Chem., Soc.,* 1871, 24, 91.
[2] *Bull. Assoc. Chim. Sucr.,* 1891, 9, 439.
[3] *Zeitsch. Ver. Zuckerind.,* 40, 1037.
[4] *D. Zuckerind.,* 23, 1753.

van Ekenstein[1] by the action of lead hydroxide on lævulose isolated a hexose, $C_6H_{12}O_6$, to which they gave the name *glutose;* it has practically no action on the plane of polarised light and is not fermentable by yeast.

The higher the temperature the more rapid is the disappearance of lævulose, so that heating a solution containing reducing sugars with basic lead acetate must always be avoided.

It is well known that Pellet[2] advocates the use of neutral lead acetate in place of the basic lead acetate in clarifying liquors which contain reducing sugars, owing to the supposed precipitating effect of the basic salt on the lævulose. But in the majority of cases the neutral lead acetate is far less effective as a clarifying agent and, in the case of plant material, frequently leaves in solution optically active substances (for example gums) which are thrown down by the basic salt and therefore prevented from interfering with the analysis. From the results obtained by the writer it would appear that if the basic lead acetate is added carefully in small quantities at a time until the precipitation of the impurities is *just* complete,[3] and if the actual excess of basic lead acetate is not allowed to exceed about 5 c.c. on 300 to 500 c.c. of solution, there is practically *no loss whatever of lævulose,* or other reducing sugars. Direct experiment with mixtures of pure reducing sugars and tannin have shown that if care be taken *to avoid any considerable excess of basic lead acetate during the precipitation,* there is no loss of reducing sugars when the tannin is precipitated in this way. Parkin[4] cites a case of this kind when the following results were obtained in a test analysis:

	Tannin added and pre-cipitated by 5 c.c. basic lead acetate	Control in distilled water
Saccharose	0.487	0.489
Dextrose	0.204	0.201
Lævulose	0.244	0.244
Total sugar	0.935	0.934

In this case, tannin was added to the solution just to the extent that it was all carried down in the precipitate caused by the addition of 5 c.c. of the basic lead solution; the excess of lead was not removed, and it is seen that this slight excess has had no influence on the result.

In agreement with the view the writer puts forward that no loss of lævulose ever occurs by actual precipitation, but that when such appears to take place it is usually caused by a transformation of the sugar by the alkaline lead solution, are the facts recently recorded by Le Docte.[5] The latter shows that whereas in carrying out the hot aqueous digestion process of extracting sugars in presence of basic lead acetate the polarisation due to lævulose (which was purposely added) *disappears* entirely, it is not changed at

[1] *Rec. Trav. Chim.,* 16, 262.
[2] *J. Fab. Sucre.,* 1899, 40, No. 15; *Bull. Assoc. Chim. Sucr.,* 1896, 15, 605; 1897, 16, 1007 and 1147; 1904, 22, 744; 1913, 31, 205; 1914, 32, 909.
[3] It is usually quite easy by making tests of small portions of the filtrate to hit off the point at which this occurs within 1 or 2 c.c., even when relatively large quantities (for example 100 to 300 c.c. of basic lead acetate have to be employed.
[4] *Biochem. J.,* 1911, 6, 12.
[5] *Sucrerie Belge,* 1912, 275.

all or changed only very slightly when the digestion is effected in the cold. Similarly when the actual hot digestion is carried out in the absence of basic lead acetate, and the latter is then added *after cooling*, the polarisation due to lævulose (or dextrose) is not in the least interfered with. It is concluded that, as stated above, when reducing sugars are present basic lead acetate should never be added to the hot solution.

Lævulose is far more sensitive to the action of basic lead acetate in the cold than dextrose or other sugars just as it is in general far more easily decomposed than these (compare page 53, action of hydrochloric acid). The writer finds that dextrose and maltose remain practically unchanged in presence of considerable excess of basic lead acetate and Le Docte (*loc. cit.*) also states that in presence of dextrose, the hot digestion with basic lead acetate can be carried out without loss of the reducing sugar. The fact that no precipitation or loss of either dextrose or maltose occurs in presence of basic lead acetate greatly simplifies the analysis in the case of solutions containing these substances. The writer has shown, for example,[1] that basic lead acetate can be used in removing impurities in the estimation of starch by means of taka-diastase (see page 74), which converts the starch quantitatively into a mixture of maltose and dextrose, without any loss whatever occurring of either of these sugars.

In sugar analysis any considerable excess of basic lead acetate should always be avoided, and, in the majority of cases, any such excess should be removed before taking the actual polarisation readings, either by means of sulphurous acid as recommended by Pellet or by sodium carbonate, sodium sulphate, etc. If any excess of basic lead acetate is left it combines with the reducing sugars (dextrose, lævulose, maltose) forming *soluble* lead compounds which have an entirely different rotation from the sugars themselves; this is particularly the case with lævulose (or invert sugar) the negative rotation of which may become positive in presence of excess of basic lead acetate.

Incomplete Inversion caused by the use of Large Quantities of Basic Lead Acetate or by the Presence of Salts of Organic Acids.—In the ordinary analysis of sugar works materials and products the quantity of basic lead acetate used is generally relatively small and as much as 5 c.c. of the lead solution can be present in excess without interfering with the completeness of the inversion under Herzfeld conditions (see Pellet, *Dosage du Sucre*, page 39). On the other hand, in working with vegetable extracts, when much larger quantities of basic lead acetate have to be used, the proportion of lead which remains unprecipitated in the solution may be so great as to lead to incomplete inversion if only the usual quantity of hydrochloric acid is employed in the Herzfeld method; even if this lead be precipitated by sodium carbonate or sulphate, sodium acetate is formed, which inhibits more or less completely, the invertive action of the hydrochloric acid

[1] *J. Agric. Sci.*, 1914, **6**, 152.

just as it does that of citric acid (see page 48). In such cases serious error in the analysis may arise and Pellet cites an instance (Private Communication) in which 6% of raffinose was returned as being present in a wild beet from this cause. Care should be taken in such cases to make the solution very faintly acid to methyl-orange by the addition of sulphuric or hydrochloric acid before adding the quantity of acid (5 c.c.) required under Herzfeld conditions. Duplicate experiments can also be profitably made in which the period of heating is prolonged to 10 or 15 minutes; Pellet has shown that under Herzfeld conditions the time of heating can be prolonged to 20 minutes (at 70°) without affecting the accuracy of the results; on the other hand, it is not desirable to increase the quantity of hydrochloric acid used to 10 c.c., as the extra proportion of acid used sensibly modifies the rotatory power of the invert sugar present.

When working with plant material or plant extracts, in the purification of which relatively large quantities of basic lead acetate have been employed, it is always advisable to carry out a control estimation of the cane sugar by means of invertase, inversion by which is complete even when sodium acetate is present in considerable quantity. It must be noted that if maltose is present, there is risk of bringing about considerable hydrolysis of this sugar to dextrose, if the time of heating be prolonged, under the Herzfeld conditions by 5 minutes. Even with 5 minutes heating, as pointed out on page 48, marked hydrolysis occurs, so that when maltose (or similar glucosides) are present it is advisable to use citric acid for the inversion under the conditions already defined (page 48).

Estimation of Raffinose.—In Vol. I, page 313 the Creydt formula

$$S = \frac{0.5188\,P - I}{0.8454}$$

is given; this is still official in the United States (*Bulletin* 107 (*revised*). 1912), but in Europe Herzfeld's later formula

$$S = \frac{0.5124\,P - (-I)}{0.839}, \qquad R = \frac{P - S}{1.85}$$

is more generally adopted.

The use of this method, however, in the case of vegetable material and beet molasses may return as raffinose numbers which are far from representing the true proportion of this substance present. This is due to the presence of other optically active substances, such as the glutamic and aspartic amides, the specific rotation of which undergoes a considerable change during the process of inversion. Pellet (*Dosage du Sucre*, page 63) gives an example of a crude sugar, showing a direct polarisation of 91.10 by the ordinary method (in presence of basic lead acetate) which, when examined according to the Herzfeld method, appeared to contain 0.5% of raffinose;

when, however, the direct polarisation was taken in presence of acid, the value found was 91.55 and the conclusion drawn was that no raffinose was actually present. Other sources of error in the estimation of raffinose may be incomplete inversion of the cane sugar owing to the presence of basic lead or sodium acetate (page 51), the presence of pentoses, etc.

From the above considerations it is clear that the estimation of raffinose by the ordinary method, now in general use, gives results upon which in the majority of cases little reliance can be placed.

Estimation of Maltose.

As stated on page 24, Wein's tables showing the reducing power of maltose give results which are 5% low.

It has been frequently proposed to estimate maltose by hydrolysis with dilute hydrochloric or sulphuric acid at 100°, noting the change of cupric reducing power or specific rotatory power of the solution after allowing for the inversion of cane sugar present. Under carefully regulated conditions this method gives approximate results in the case of pure maltose or a mixture of maltose and dextrose (cf. Baker and Dick[1]) but, as shown by Davis and Daish[2] it is quite useless when saccharose or lævulose are also present, owing to the large amount of decomposition of the latter sugar which is caused by the prolonged heating with dilute acids. In the paper cited, it is shown that it is impossible to effect complete hydrolysis of maltose by dilute acid at 100°, without at the same time destroying a large proportion of the lævulose which is present; thus 31% of the lævulose present in a 1% solution is destroyed when this solution is heated with 2.4% hydrochloric acid during 2 hours, this being the time necessary to effect 98% of complete hydrolysis of maltose in a 1 per cent. solution of this sugar. Even at 70° it is impossible completely to hydrolyse maltose in presence of cane sugar or lævulose without at the same time destroying considerable quantities of lævulose; thus in 1% solution of the sugar, even after 24 hours' heating with a 2.4% solution of hydrochloric acid only 94% of the maltose is hydrolysed, whilst more than 5% of the lævulose is destroyed.

It is therefore impossible to estimate maltose with any degree of accuracy by acid hydrolysis when cane sugar or lævulose are also present. In such cases it is necessary to employ a biochemical method, involving the use of maltase-free yeasts. Davis and Daish (*loc. cit.*) have suggested the following process, which has been thoroughly tested and found to give quite accurate results; it was shown that it is possible to ferment away every trace of dextrose, lævulose, or cane sugar by means of any one of the three special yeasts *Saccharomyces marxianus, S. exiguus,* or *S. anomalus* and to recover every trace

[1] *Analyst,* 1905, 30, 79.
[2] *J. Agric. Sci.,* 1913, 5, 453.

of maltose, which can then be estimated by measuring the residual reducing power.

To 50 c.c. of the solution containing the sugars (previously purified by means of basic lead acetate and then freed from lead as described below) which must be very faintly acid to litmus paper, 5 c.c. of yeast water is added; the solution is then sterilised in a flask closed with a cotton-wool stopper, by heating during 20 minutes in the autoclave at 115–120°, and is then inoculated with a trace of the pure maltase-free yeast and incubated at 25° for 3 to 4 weeks. When the fermentation is complete[1] 5 c.c. of alumina cream are added and the solution well boiled; it is then filtered and the precipitate well washed until the filtrate has a volume of 100 c.c. An aliquot portion (50 c.c.) can then be used for measuring the reducing power.

For the yeasts to grow satisfactorily it is necessary to make sure that every trace of lead has been removed from the solution. The best method of effecting this is to throw down the excess of lead by adding sodium carbonate, little by little until no further precipitate is produced, then to make the filtrate slightly acid with hydrochloric acid and to precipitate the traces of lead remaining in solution by hydrogen sulphide. The lead can also be removed directly from the solution by using hydrogen sulphide without the prior employment of sodium carbonate, but in such cases the solution usually becomes strongly acid if much lead acetate be present (owing to the liberation of acetic acid) and this acidity must be very nearly neutralised by adding sodium carbonate before sterilising and inoculating with the yeast. In all cases, hydrogen sulphide is expelled by sucking air through the solution obtained after filtering from the lead sulphide; the solution which is used for the fermentations should show a faint but distinct acid indication to litmus.

When small quantities of pentoses are present in the solutions to be analysed (as is frequently the case in dealing with plant extracts) it is necessary to introduce a correction for these; the pentoses are very slowly, if at all, fermented by the maltase-free yeasts and consequently, like maltose itself, exercise a reducing effect on the Fehling solution. The correction for the pentoses is obtained by carrying out fermentations with a pure culture of ordinary distillery or brewers' yeast which ferments away the maltose but leaves the pentoses; the slight residual reducing power found after carrying out a fermentation with such yeast is indeed a measure of the pentoses present and can be used as a means of verifying the presence of these substances or of estimating them in cases when the ordinary pentose method is likely to give incorrect results owing to the presence of other sugars (Davis and Sawyer).[2] On subtracting the value found for the reducing power remaining after fermentation with ordinary yeast from the value given by the maltase-free yeasts, the cupric reduction due to the maltose alone is obtained.

[1] 0.20 to 0.5 grm. of cane sugar are invariably completely fermented under the above conditions after 3 weeks.
[2] J. Agric. Sci., 1914, 6, 406.

During the past three years the writer has used this method of estimating maltose in more than 500 analyses of various kinds of plant material (plant extracts, germinated and ungerminated grain, starch conversions, etc.). It has been our custom to carry out five fermentations with each solution to be analysed, viz., one each with *S. anomalus*, *S. exiguus*, and *S. marxianus*, and two with distillery yeast. The agreement between the results with the different special yeasts has generally been entirely satisfactory. *S. anomalus* does not lend itself quite so well to quantitative experiments as the other maltase-free yeasts, as it is slower in its action and is less efficient as a sugar-remover—that is, a greater growth of yeast is necessary for the removal of a certain weight of sugar. Moreover, the Fehling solution in the subsequent reduction often filters very slowly and the cuprous oxide is also generally somewhat contaminated by traces of copper compounds formed by the action of the Fehling solution on substances elaborated during the growth of the yeast. It is therefore best to make use of *S. exiguus* and *S. marxianus* only. On page 64 is given a general scheme for the analysis of plant material, showing the way in which this method is applied in such cases.

Biochemical Methods of Estimating Sugars.—The estimation of maltose in the presence of other sugars dealt with above is an instance in which the ordinary chemical processes of sugar estimation are entirely useless and in which biochemical methods have to be employed. Kluyver has independently applied somewhat similar principles, with considerable success, to the estimation of the different constituent sugars present in complex mixtures of sugars, for example of maltose, saccharose, raffinose, with the simple hexoses. Full details are given in the monograph *Biochemische Suikerbepalingen* (Leiden, E. J. Brill, 1914. Kluyver makes use of a gasometric method, measuring the volume of carbon dioxide evolved by the action of a series of special yeasts and torulæ upon the solution dealt with; this method has the advantage that approximately accurate results are obtained with exceedingly small quantities of the solutions or sugar dealt with. A special eudiometer was devised in which the fermentation is carried out and the gas measured. A special case may be cited to illustrate the principle of applying this process, the sugars present, for example, being raffinose, cane sugar, and monoses; the ferments used were *S. cerevisiæ* (under-yeast U), ordinary press yeast, *Torula dattila* and *Torula monosa*. The difference between the results with the first two yeasts gives the raffinose; the cane sugar can then be calculated from the volume of gas given by *Torula dattila*, after allowing for the raffinose present, whilst the gas evolved by *Torula monosa* is a measure of the hexoses present (dextrose, lævulose or mannose), as only these sugars are fermented by this organism. The process is said to be relatively rapid and to give approximately accurate results in cases where other methods are inapplicable. Biochemical methods have been applied

[1] *Ann. Inst. Pasteur*, 1906, 20, 977.

by Kluyver to the analysis of food materials such as jams, potato syrups and to other cases of technical importance.

The experiments of Bertrand and Weisweiller[1] and Bertrand and Duchàček[1] have shown that the Bulgarian bacillus (*Bacillus acidi lactici Massol*) is capable of converting hexoses such as *d*-glucose, lævulose, mannose and galactose into lactic acid, but acts upon only one of the disaccharides in this way, namely, lactose. Saccharose and maltose are not attacked by this organism, and Margaillan suggested that the latter should be used as a means of separating cane sugar from lactose and *d*-glucose. Testoni[2] has worked out a process of estimating saccharose in condensed milk and similar materials based on this principle. 20 grm. of condensed milk are dissolved in warm water and mixed with acetic acid to coagulate proteins, and then with basic lead acetate, the excess of lead being removed by means of a saturated solution of sodium sulphate. The solution is made up to 200 c.c., and an additional 2 c.c. of water are added to compensate for the volume of the coagulum. 100 c.c. of the filtrate are mixed with malt-peptone and 3 grm. of powdered marble, neutralised exactly, using phenolphthaleïn as indicator, and sterilised. The solution is inoculated with a pure culture of the bacillus and left for 6 days in an incubator at 35°. The lactic acid formed is precipitated by adding a slight excess of zinc sulphate and the volume made up to 100 c.c.; the solution is quickly filtered and the rotatory power measured with the polarimeter.

Jolles[3] has stated that arabinose, rhamnose, *d*-glucose, fructose, galactose, mannose, invert sugar, maltose and lactose are completely decomposed when heated with $N/10$ sodium hydroxide during 45 minutes in the water-bath or during 24 hours in a thermostat at 37°, acid substances being formed which are without action on polarised light; saccharose, on the other hand, is not in the least affected by this treatment, so that it is possible to estimate this substance in presence of the above-named sugars by treatment with sodium hydroxide under the conditions named. Testoni states that Jolles' method gives the same results with sweetened wines, condensed milk and marmalades as are obtained by his process of fermentation[4] and as the treatment with sodium hydroxide can be carried out rapidly, Jolles' method is preferable in most cases to the more tedious fermentation process. It is best to work at 37° rather than 100° as at the lower temperature darkening of the solution and the consequent difficulty of reading the rotatory power of the solution are avoided. Before measuring the rotation the solution should be neutralised or made slightly acid with acetic acid, as otherwise the readings are slightly low. The nature of the change of rotation brought about by treatment with sodium hydroxide indicates whether commercial glucose or invert sugar was originally present. Bardach and Silberstein[5] have also applied Jolles' process to a number of commercial preparations.

[1] *Ann. Inst. Pasteur*, 1909, 23, 402.
[2] *Ann. Lab. Centr. delle Gabelle*, 1912, page 581.
[3] *Zeit. Nahr. und Genussm.*, 1910, 20, 631.
[4] Compare Nowak, *Zeit. Anal. Chem.*, 1912, 51, 610.
[5] *Zeit. Nahr. und Genussm.*, 1911, 21, 540.

Lactose.

Cole[1] has given the following table for lactose. He makes use of the iodometric method in estimating the copper, under the following conditions: Into a 200 c.c. Erlenmeyer flask measure 20 c.c. of standardised copper sulphate, 20 c.c. of the alkaline copper tartrate and 20 c.c. of the sugar solution to be tested (which must contain between 5 and 250 mm. of anhydrous lactose). Fit a two-holed rubber stopper firmly into the neck of the flask, adjust a thermometer so that its lower end is 2 mm. from the bottom of the flask and place the latter on the heated gauze. Note the time when the mercury indicates a temperature of 95° and allow the heating to continue for exactly 20 seconds beyond this. Remove the flask by gripping the rubber stopper and rapidly cool under the tap. Filter the hot solution at once, using the stem of the thermometer as a stirring rod. Wash the flask twice with about 7 c.c. of distilled water, cool the filtrate under the tap, add exactly 4 c.c. of concentrated sulphuric acid, insert a thermometer and cool to 20°. Add 6.5 to 7 c.c. of a saturated solution of potassium iodide, washing the stem of the thermometer with this solution. Titrate at once with the standardised solution of sodium thiosulphate in the usual way, using soluble starch as indicator.[2]

TABLE VIII.—REDUCING POWER OF LACTOSE (*Cole.*)

Anhydrous lactose	Copper mg.	Lactose: copper
5	4.4	1.136
10	9.8	1.020
15	16.6	0.903
20	24.1	0.830
25	30.5	0.820
30	36.9	0.813
35	43.2	0.810
40	49.9	0.802
45	56.1	0.802
50	62.5	0.800
55	69.0	0.805
60	75.2	0.798
65	81.3	0.799
70	88.2	0.793
75	94.0	0.798
80	100.9	0.793
85	107.3	0.792
90	113.6	0.792
95	120.1	0.791
100	127.0	0.788
120	152.8	0.786
130	166.1	0.783
140	179.4	0.780
150	191.6	0.783
175	223.0	0.785
200	255.5	0.782
240	307.3	0.781
250	320.7	0.780

Below are given the values for lactose which are employed provisionally in the United States by the A. O. A. C.; they were obtained by the gravimetric method described on page 28, and supplement the tables given on pages 29 to 37 for other sugars.

[1] *Biochem. J.*, 1914, 8, 134.
[2] Cole attributes the method of estimating sugars iodometrically to Peters (*J. Amer. Chem. Soc.*, 1912, 34, 422 and 928). Schoorl in 1899 suggested substantially the same process (see page 41) and gave tables of reducing values.

TABLE IX.—TABLE FOR CALCULATING LACTOSE.

(Correction for *Bulletin* 107 (*revised*), pages 243–251.)

(From *Circular* 82, U. S. Dept. of Agric., Dec. 30, 1911.)

(Expressed in milligrams.)

Cuprous oxide (Cu₂O)	Copper (Cu)	Lactose			Cuprous oxide (Cu₂O)	Copper (Cu)	Lactose		
		(C₁₂H₂₂O₁₁)	(C₁₂H₂₂O₁₁ ½H₂O)	(C₁₂H₂₂O₁₁,H₂O)			(C₁₂H₂₂O₁₁)	(C₁₂H₂₂O₁₁ ½H₂O)	(C₁₂H₂₂O₁₁,H₂O)
10	8.9	3.8	3.9	4.0	65	57.7	40.0	41.0	42.1
11	9.8	4.5	4.6	4.7	66	58.6	40.6	41.7	42.8
12	10.7	5.1	5.3	5.4	67	59.5	41.3	42.4	43.5
13	11.5	5.8	5.9	6.1	68	60.4	41.9	43.1	44.2
14	12.4	6.4	6.6	6.8	69	61.3	42.6	43.7	44.8
15	13.3	7.1	7.3	7.5	70	62.2	43.3	44.4	45.5
16	14.2	7.8	8.0	8.2	71	63.1	43.9	45.1	46.2
17	15.1	8.4	8.6	8.9	72	64.0	44.6	45.8	46.9
18	16.0	9.1	9.3	9.5	73	64.8	45.2	46.4	47.6
19	16.9	9.7	10.0	10.2	74	65.7	45.9	47.1	48.3
20	17.8	10.4	10.7	10.9	75	66.6	46.6	47.8	49.0
21	18.7	11.0	11.3	11.6	76	67.5	47.2	48.5	49.7
22	19.5	11.7	12.0	12.3	77	68.4	47.9	49.1	50.4
23	20.4	12.3	12.7	13.0	78	69.3	48.5	49.8	51.1
24	21.3	13.0	13.4	13.7	79	70.2	49.2	50.5	51.8
25	22.2	13.7	14.0	14.4	80	71.1	49.9	51.2	52.5
26	23.1	14.3	14.7	15.1	81	71.9	50.5	51.9	53.2
27	24.0	15.0	15.4	15.8	82	72.8	51.2	52.5	53.9
28	24.9	15.6	16.1	16.5	83	73.7	51.8	53.2	54.6
29	25.8	16.3	16.7	17.1	84	74.6	52.5	53.9	55.3
30	26.6	16.9	17.4	17.8	85	75.5	53.1	54.6	56.0
31	27.5	17.6	18.1	18.5	86	76.4	53.8	55.2	56.6
32	28.4	18.3	18.7	19.2	87	77.3	54.5	55.9	57.3
33	29.3	18.9	19.4	19.9	88	78.2	55.1	56.6	58.0
34	30.2	19.6	20.1	20.6	89	79.1	55.8	57.3	58.7
35	31.1	20.2	20.8	21.3	90	79.9	56.4	58.0	59.4
36	32.0	20.9	21.4	22.0	91	80.8	57.1	58.6	60.1
37	32.9	21.5	22.1	22.7	92	81.7	57.8	59.3	60.8
38	33.8	22.2	22.8	23.4	93	82.6	58.4	60.0	61.5
39	34.6	22.8	23.5	24.1	94	83.5	59.1	60.7	62.2
40	35.5	23.5	24.1	24.8	95	84.4	59.7	61.3	62.9
41	36.4	24.2	24.8	25.4	96	85.3	60.4	62.0	63.6
42	37.3	24.8	25.5	26.1	97	86.2	61.1	62.7	64.3
43	38.2	25.5	26.2	26.8	98	87.0	61.7	63.4	65.0
44	39.1	26.1	26.8	27.5	99	87.9	62.4	64.0	65.7
45	40.0	26.8	27.5	28.2	100	88.8	63.0	64.7	66.4
46	40.9	27.4	28.2	28.9	101	89.7	63.7	65.4	67.1
47	41.7	28.1	28.9	29.6	102	90.6	64.4	66.1	67.8
48	42.6	28.7	29.5	30.3	103	91.5	65.0	66.7	68.5
49	43.5	29.4	30.2	31.0	104	92.4	65.7	67.4	69.1
50	44.4	30.1	30.9	31.7	105	93.3	66.4	68.1	69.8
51	45.3	30.7	31.5	32.4	106	94.2	67.0	68.8	70.5
52	46.2	31.4	32.2	33.0	107	95.0	67.7	69.5	71.2
53	47.1	32.1	32.9	33.7	108	95.9	68.3	70.1	71.9
54	48.0	32.7	33.6	34.4	109	96.8	69.0	70.8	72.6
55	48.9	33.4	34.3	35.1	110	97.7	69.7	71.5	73.3
56	49.7	34.0	34.9	35.8	111	98.6	70.3	72.2	74.0
57	50.6	34.7	35.6	36.5	112	99.5	71.0	72.8	74.7
58	51.5	35.4	36.3	37.2	113	100.4	71.6	73.5	75.4
59	52.4	36.0	37.0	37.9	114	101.3	72.3	74.2	76.1
60	53.3	36.7	37.6	38.6	115	102.1	73.0	74.9	76.8
61	54.2	37.3	38.3	39.3	116	103.0	73.6	75.6	77.5
62	55.1	38.0	39.0	40.0	117	103.9	74.3	76.2	78.2
63	56.0	38.6	39.7	40.7	118	104.8	75.0	76.9	78.9
64	56.8	39.3	40.3	41.4	119	105.7	75.6	77.6	79.6

TABLE IX.—TABLE FOR CALCULATING LACTOSE.—*Continued.*

Cuprous oxide (Cu₂O)	Copper (Cu)	Lactose ($C_{12}H_{22}O_{11}$)	Lactose ($C_{12}H_{22}O_{11}$ ½H_2O)	Lactose ($C_{12}H_{22}O_{11},H_2O$)	Cuprous oxide (Cu₂O)	Copper (Cu)	Lactose ($C_{12}H_{22}O_{11}$)	Lactose ($C_{12}H_{22}O_{11}$ ½H_2O)	Lactose ($C_{12}H_{22}O_{11},H_2O$)
120	106.6	76.3	78.3	80.3	180	159.9	116.1	119.1	122.2
121	107.5	76.9	79.0	81.0	181	160.8	116.7	119.8	122.9
122	108.4	77.6	79.6	81.7	182	161.7	117.4	120.5	123.6
123	109.3	78.3	80.3	82.4	183	162.5	118.1	121.2	124.3
124	110.1	78.9	81.0	83.1	184	163.4	118.7	121.8	125.0
125	111.0	79.6	81.7	83.8	185	164.3	119.4	122.5	125.7
126	111.9	80.3	82.4	84.5	186	165.2	120.1	123.2	126.4
127	112.8	80.9	83.0	85.2	187	166.1	120.7	123.9	127.1
128	113.7	81.6	83.7	85.9	188	167.0	121.4	124.6	127.8
129	114.6	82.2	84.4	86.6	189	167.9	122.1	125.3	128.5
130	115.5	82.9	85.1	87.3	190	168.8	122.7	125.9	129.2
131	116.4	83.6	85.7	88.0	191	169.7	123.4	126.6	129.9
132	117.2	84.2	86.4	88.7	192	170.5	124.1	127.3	130.6
133	118.1	84.9	87.1	89.4	193	171.4	124.7	128.0	131.3
134	119.0	85.5	87.8	90.1	194	172.3	125.4	128.7	132.0
135	119.9	86.2	88.5	90.8	195	173.2	126.1	129.4	132.7
136	120.8	86.9	89.1	91.5	196	174.1	126.7	130.0	133.4
137	121.7	87.5	89.8	92.1	197	175.0	127.4	130.7	134.1
138	122.6	88.2	90.5	92.8	198	175.9	128.1	131.4	134.8
139	123.5	88.9	91.2	93.5	199	176.8	128.7	132.1	135.5
140	124.4	89.5	91.9	94.2	200	177.6	129.4	132.8	136.2
141	125.2	90.2	92.5	94.9	201	178.5	130.0	133.5	136.9
142	126.1	90.8	93.2	95.6	202	179.4	130.7	134.1	137.6
143	127.0	91.5	93.9	96.3	203	180.3	131.4	134.8	138.3
144	127.9	92.2	94.6	97.0	204	181.2	132.0	135.5	139.0
145	128.8	92.8	95.3	97.7	205	182.1	132.7	136.2	139.7
146	129.7	93.5	95.9	98.4	206	183.0	133.4	136.9	140.4
147	130.6	94.2	96.6	99.1	207	183.9	134.0	137.6	141.1
148	131.5	94.8	97.3	99.8	208	184.8	134.7	138.3	141.8
149	132.3	95.5	98.0	100.5	209	185.6	135.4	138.9	142.5
150	133.2	96.1	98.7	101.2	210	186.5	136.0	139.6	143.2
151	134.1	96.8	99.3	101.9	211	187.4	136.7	140.3	143.9
152	135.0	97.5	100.0	102.6	212	188.3	137.4	141.0	144.6
153	135.9	98.1	100.7	103.3	213	189.2	138.0	141.7	145.3
154	136.8	98.8	101.4	104.0	214	190.1	138.7	142.4	146.0
155	137.7	99.5	102.1	104.7	215	191.0	139.4	143.0	146.7
156	138.6	100.1	102.8	105.4	216	191.9	140.0	143.7	147.4
157	139.5	100.8	103.4	106.1	217	192.7	140.7	144.4	148.1
158	140.3	101.5	104.1	106.8	218	193.6	141.4	145.1	148.8
159	141.2	102.1	104.8	107.5	219	194.5	142.0	145.8	149.5
160	142.1	102.8	105.5	108.2	220	195.4	142.7	146.5	150.2
161	143.0	103.4	106.2	108.9	221	196.3	143.4	147.2	150.9
162	143.9	104.1	106.8	109.6	222	197.2	144.0	147.8	151.6
163	144.8	104.8	107.5	110.3	223	198.1	144.7	148.5	152.3
164	145.7	105.4	108.2	111.0	224	199.0	145.4	149.2	153.0
165	146.6	106.1	108.9	111.7	225	199.8	146.0	149.9	153.7
166	147.4	106.8	109.6	112.4	226	200.7	146.7	150.6	154.4
167	148.3	107.4	110.3	113.1	227	201.6	147.4	151.3	155.1
168	149.2	108.1	110.9	113.8	228	202.5	148.0	152.0	155.8
169	150.1	108.8	111.6	114.5	229	203.4	148.7	152.6	156.5
170	151.0	109.4	112.3	115.2	230	204.3	149.4	153.3	157.2
171	151.9	110.1	113.0	115.9	231	205.2	150.0	154.0	157.9
172	152.8	110.8	113.7	116.6	232	206.1	150.7	154.7	158.6
173	153.7	111.4	114.3	117.3	233	207.0	151.4	155.4	159.3
174	154.6	112.1	115.0	118.0	234	207.8	152.0	156.1	160.0
175	155.4	112.8	115.7	118.7	235	208.7	152.7	156.7	160.7
176	156.3	113.4	116.4	119.4	236	209.6	153.4	157.4	161.4
177	157.2	114.1	117.1	120.1	237	210.5	154.0	158.1	162.1
178	158.1	114.8	117.8	120.8	238	211.4	154.7	158.8	162.8
179	159.0	115.4	118.4	121.5	239	212.3	155.4	159.5	163.5

TABLE IX.—TABLE FOR CALCULATING LACTOSE.—*Continued.*

Cuprous oxide (Cu₂O)	Copper (Cu)	Lactose ($C_{12}H_{22}O_{11}$)	Lactose ($C_{12}H_{22}O_{11}$ ½H₂O)	Lactose ($C_{12}H_{22}O_{11}$, H₂O)	Cuprous oxide (Cu₂O)	Copper (Cu)	Lactose ($C_{12}H_{22}O_{11}$)	Lactose ($C_{12}H_{22}O_{11}$ ½H₂O)	Lactose ($C_{12}H_{22}O_{11}$, H₂O)
240	213.2	156.1	160.2	164.3	305	270.9	199.6	204.9	210.1
241	214.1	156.7	160.9	165.0	306	271.8	200.3	205.5	210.8
242	214.9	157.4	161.5	165.7	307	272.7	201.0	206.2	211.5
243	215.8	158.1	162.2	166.4	308	273.6	201.6	206.9	212.2
244	216.7	158.7	162.9	167.1	309	274.5	202.3	207.6	212.9
245	217.6	159.4	163.6	167.8	310	275.3	203.0	208.3	213.7
246	218.5	160.1	164.3	168.5	311	276.2	203.6	209.0	214.4
247	219.4	160.7	165.0	169.2	312	277.1	204.3	209.7	215.1
248	220.3	161.4	165.7	169.9	313	278.0	205.0	210.4	215.8
249	221.2	162.1	166.3	170.6	314	278.9	205.7	211.1	216.5
250	222.1	162.7	167.0	171.3	315	279.8	206.3	211.8	217.2
251	222.9	163.4	167.7	172.0	316	280.7	207.0	212.5	217.9
252	223.8	164.1	168.4	172.7	317	281.6	207.7	213.1	218.6
253	224.7	164.7	169.1	173.4	318	282.5	208.4	213.8	219.3
254	225.6	165.4	169.8	174.1	319	283.3	209.0	214.5	220.0
255	226.5	166.1	170.5	174.8	320	284.2	209.7	215.2	220.7
256	227.4	166.8	171.1	175.5	321	285.1	210.4	215.9	221.4
257	228.3	167.4	171.8	176.2	322	286.0	211.0	216.6	222.2
258	229.2	168.1	172.5	176.9	323	286.9	211.7	217.3	222.9
259	230.0	168.8	173.2	177.6	324	287.8	212.4	218.0	223.6
260	230.9	169.4	173.9	178.3	325	288.7	213.1	218.7	224.3
261	231.8	170.1	174.6	179.0	326	289.6	213.7	219.4	225.0
262	232.7	170.8	175.3	179.8	327	290.4	214.4	220.1	225.7
263	233.6	171.4	176.0	180.5	328	291.3	215.1	220.7	226.4
264	234.5	172.1	176.6	181.2	329	292.2	215.8	221.4	227.1
265	235.4	172.8	177.3	181.9	330	293.1	216.4	222.1	227.8
266	236.3	173.5	178.0	182.6	331	294.0	217.1	222.8	228.5
267	237.2	174.1	178.7	183.3	332	294.9	217.8	223.5	229.2
268	238.0	174.8	179.4	184.0	333	295.8	218.4	224.2	230.0
269	238.9	175.5	180.1	184.7	334	296.7	219.1	224.9	230.7
270	239.8	176.1	180.8	185.4	335	297.6	219.8	225.6	231.4
271	240.7	176.8	181.5	186.1	336	298.4	220.5	226.3	232.1
272	241.6	177.5	182.1	186.8	337	299.3	221.1	227.0	232.8
273	242.5	178.1	182.8	187.5	338	300.2	221.8	227.7	233.5
274	243.4	178.8	183.5	188.2	339	301.1	222.5	228.3	234.2
275	244.3	179.5	184.2	188.9	340	302.0	223.2	229.0	234.9
276	245.1	180.2	184.9	189.6	341	302.9	223.8	229.7	235.6
277	246.0	180.8	185.6	190.3	342	303.8	224.5	230.4	236.3
278	246.9	181.5	186.3	191.0	343	304.7	225.2	231.1	237.0
279	247.8	182.2	187.0	191.7	344	305.5	225.9	231.8	237.8
280	248.7	182.8	187.7	192.4	345	306.4	226.5	232.5	238.5
281	249.6	183.5	188.3	193.1	346	307.3	227.2	233.2	239.2
282	250.5	184.2	189.0	193.9	347	308.2	227.9	233.9	239.9
283	251.4	184.8	189.7	194.6	348	309.1	228.5	234.6	240.6
284	252.3	185.5	190.4	195.3	349	310.0	229.2	235.3	241.3
285	253.1	186.2	191.1	196.0	350	310.9	229.9	235.9	242.0
286	254.0	186.9	191.8	196.7	351	311.8	230.6	236.6	242.7
287	254.9	187.5	192.5	197.4	352	312.7	231.2	237.3	243.4
288	255.8	188.2	193.2	198.1	353	313.5	231.9	238.0	244.1
289	256.7	188.9	193.8	198.8	354	314.4	232.6	238.7	244.8
290	257.6	189.5	194.5	199.5	355	315.3	233.3	239.4	245.6
291	258.5	190.2	195.2	200.2	356	316.2	233.9	240.1	246.3
292	259.4	190.9	195.9	200.9	357	317.1	234.6	240.8	247.0
293	260.2	191.5	196.6	201.6	358	318.0	235.3	241.5	247.7
294	261.1	192.2	197.3	202.3	359	318.9	236.0	242.2	248.4
295	262.0	192.9	198.0	203.0	360	319.8	236.7	242.9	249.1
296	262.9	193.6	198.7	203.7	361	320.6	237.3	243.6	249.8
297	263.8	194.2	199.3	204.4	362	321.5	238.0	244.3	250.5
298	264.7	194.9	200.0	205.1	363	322.4	238.7	245.0	251.2
299	265.6	195.6	200.7	205.8	364	323.3	239.4	245.7	252.0
300	266.5	196.2	201.4	206.6	365	324.2	240.0	246.4	252.7
301	267.4	196.9	202.1	207.3	366	325.1	240.7	247.0	253.4
302	268.2	197.6	202.8	208.0	367	326.0	241.4	247.7	254.1
303	269.1	198.3	203.5	208.7	368	326.9	242.1	248.4	254.8
304	270.0	198.9	204.2	209.4	369	327.8	242.7	249.1	255.5

TABLE IX.—TABLE FOR CALCULATING LACTOSE.—*Continued.*

Cuprous oxide (Cu₂O)	Copper (Cu)	Lactose			Cuprous oxide (Cu₂O)	Copper (Cu)	Lactose		
		($C_{12}H_{22}O_{11}$)	($C_{12}H_{22}O_{11}$ ½H₂O)	($C_{12}H_{22}O_{11}$, H₂O)			($C_{12}H_{22}O_{11}$)	($C_{12}H_{22}O_{11}$ ½H₂O)	($C_{12}H_{22}O_{11}$, H₂O)
370	328.6	243.4	249.8	256.2	430	381.9	284.1	291.5	299.0
371	329.5	244.1	250.5	256.9	431	382.8	284.7	292.2	299.7
372	330.4	244.8	251.2	257.7	432	383.7	285.4	292.9	300.5
373	331.3	245.4	251.9	258.4	433	384.6	286.1	293.6	301.2
374	332.2	246.1	252.6	259.1	434	385.5	286.8	294.3	301.9
375	333.1	246.8	253.3	259.8	435	386.4	287.5	295.0	302.6
376	334.0	247.5	254.0	260.5	436	387.3	288.1	295.7	303.3
377	334.9	248.1	254.7	261.2	437	388.8	288.8	296.4	304.0
378	335.7	248.8	255.4	261.9	438	389.0	289.5	297.1	304.7
379	336.6	249.5	256.1	262.6	439	389.9	290.2	297.8	305.5
380	337.5	250.2	256.8	263.4	440	390.8	290.9	298.5	306.2
381	338.4	250.8	257.5	264.1	441	391.7	291.5	299.2	306.9
382	339.3	251.5	258.1	264.8	442	392.6	292.2	299.9	307.6
383	340.2	252.2	258.8	265.5	443	393.5	292.9	300.6	308.3
384	341.1	252.9	259.5	266.2	444	394.4	293.6	301.3	309.0
385	342.0	253.6	260.2	266.9	445	395.3	294.2	302.0	309.7
386	342.9	254.2	260.9	267.6	446	396.1	294.9	302.7	310.5
387	343.7	254.9	261.6	268.3	447	397.0	295.6	303.4	311.2
388	344.6	255.6	262.3	269.0	448	397.9	296.3	304.1	311.9
389	345.5	256.3	263.0	269.8	449	398.8	297.0	304.8	312.6
390	346.4	256.9	263.7	270.5	450	399.7	297.6	305.5	313.3
391	347.3	257.6	264.4	271.2	451	400.6	298.3	306.2	314.0
392	348.2	258.3	265.1	271.9	452	401.5	299.0	306.9	314.7
393	349.1	259.0	265.8	272.6	453	402.4	299.7	307.6	315.5
394	350.0	259.6	266.5	273.3	454	403.3	300.4	308.3	316.2
395	350.8	260.3	267.2	274.0	455	404.1	301.1	309.0	316.9
396	351.7	261.0	267.9	274.7	456	405.0	301.7	309.7	317.6
397	352.6	261.7	268.6	275.5	457	405.9	302.4	310.4	318.3
398	353.5	262.3	269.3	276.2	458	406.8	303.1	311.1	319.0
399	354.4	263.0	269.9	276.9	459	407.7	303.8	311.8	319.8
400	355.3	263.7	270.6	277.6	460	408.6	304.5	312.5	320.5
401	356.2	264.4	271.3	278.3	461	409.5	305.1	313.2	321.2
402	357.1	265.0	272.0	279.0	462	410.4	305.8	313.9	321.9
403	358.0	265.7	272.7	279.7	463	411.2	306.5	314.6	322.6
404	358.8	266.4	273.4	280.4	464	412.1	307.2	315.3	323.4
405	359.7	267.1	274.1	281.1	465	413.0	307.9	316.0	324.1
406	360.6	267.8	274.8	281.9	466	413.9	308.6	316.7	324.8
407	361.5	268.4	275.5	282.6	467	414.8	309.2	317.4	325.5
408	362.4	269.1	276.2	283.3	468	415.7	309.9	318.1	326.2
409	363.3	269.8	276.9	284.0	469	416.6	310.6	318.8	326.9
410	364.2	270.5	277.6	284.7	470	417.5	311.3	319.5	327.7
411	365.1	271.2	278.3	285.4	471	418.4	312.0	320.2	328.4
412	365.9	271.8	279.0	286.2	472	419.2	312.6	320.9	329.1
413	366.8	272.5	279.7	286.9	473	420.1	313.3	321.6	329.8
414	367.7	273.2	280.4	287.6	474	421.0	314.0	322.3	330.5
415	368.6	273.9	281.1	288.3	475	421.9	314.7	323.0	331.3
416	369.5	274.6	281.8	289.0	476	422.8	315.4	323.7	332.0
417	370.4	275.2	282.5	289.7	477	423.7	316.1	324.4	332.7
418	371.3	275.9	283.2	290.4	478	424.6	316.7	325.1	333.4
419	372.2	276.6	283.9	291.2	479	425.5	317.4	325.8	334.1
420	373.1	277.3	284.6	291.9	480	426.3	318.1	326.5	334.8
421	373.9	277.9	285.3	292.6	481	427.2	318.8	327.2	335.6
422	374.8	278.6	286.0	293.3	482	428.1	319.5	327.9	336.3
423	375.7	279.3	286.7	294.0	483	429.0	320.1	328.6	337.0
424	376.6	280.0	287.4	294.7	484	429.9	320.8	329.3	337.7
					485	430.8	321.5	330.0	338.4
425	377.5	280.7	288.1	295.4	486	431.7	322.2	330.7	339.1
426	378.4	281.3	288.8	296.2	487	432.6	322.9	331.4	339.9
427	379.3	282.0	289.4	296.9	488	433.5	323.6	332.1	340.6
428	380.2	282.7	290.1	297.6	489	434.3	324.2	332.8	341.3
429	381.0	283.4	290.8	298.3	490	435.2	324.9	333.5	342.0

The method which is provisional in the United States[1] of estimating lactose and succharose in presence of one another, for example in cocoa products, is given in full in Vol. 6, page 713 (Dubois' method). As this method is purely a polarimetric one and the presence of salts and other substances in special cases may falsify the results by modifying the optical data, it is always advisable to check the results obtained by an alternative process. The lactose itself, for example, can be estimated in presence of saccharose by measuring its reducing power; this can be done either volumetrically or gravimetrically. If the volumetric process be adopted the Fehling solution should be standardised against pure lactose; and if the gravimetric method be followed tables should be used or prepared for the precise conditions under which the estimation is carried out. The cane sugar can be estimated separately by measuring the change of reducing and specific rotatory powers brought about by invertase (autolysed yeast, see page 46). In presence of fermentable sugars such as maltose, invert sugar, cane sugar, etc., lactose can be estimated by fermenting away these sugars by means of ordinary yeast and then measuring the reducing power or rotatory power of the residual material; lactose is not fermented by ordinary yeast.

Pentoses and Pentosans.

The reducing powers of pure arabinose and xylose under the standard conditions of Brown, Morris and Millar (page 24) have been determined by Daish.[2] The values obtained are given in Tables X and XI below, and refer to the anhydrous sugars.

TABLE X.—REDUCING POWER OF ARABINOSE.

$[\alpha]_D^{20°} = 102.2°$ (c = 6.80).

Milligrams, arabinose	Grams, CuO	Calculated divisor	Divisor from curve
10	0.0270	2.700	2.669
20	0.0540	2.700	2.654
30	0.0804	2.680	2.640
40	0.1064	2.660	2.625
50	0.1320	2.640	2.610
60	0.1570	2.617	2.595
70	0.1820	2.600	2.581
80	0.2060	2.575	2.566
90	0.2300	2.556	2.551
100	0.2540	2.540	2.536
110	0.2780	2.527	2.521
120	0.3020	2.517	2.507
130	0.3248	2.499	2.492
140	0.3476	2.483	2.477
150	0.3700	2.467	2.461
160	0.3920	2.450	2.447
170	0.4140	2.435	2.432
180	0.4360	2.422	2.417
190	0.4570	2.405	2.403
200	0.4780	2.390	2.381

[1] Bulletin 107 (revised), 1912, p. 256.
[2] J. Agric. Sci., 1914, 6, 225.

TABLE XI.—REDUCING POWER OF XYLOSE.

$$[\alpha]_D^{20°} = 18.78° \ (c = 5.07)$$

Milligrams, xylose	Grams, CuO	Calculated divisor	Divisor from curve
10	0.0280	2.800	2.656
20	0.0540	2.700	2.638
30	0.0798	2.660	2.620
40	0.1040	2.600	2.602
50	0.1300	2.600	2.581
60	0.1540	2.583	2.563
70	0.1790	2.557	2.545
80	0.2030	2.537	2.526
90	0.2260	2.511	2.508
100	0.2490	2.490	2.490
110	0.2720	2.473	2.471
120	0.2940	2.450	2.453
130	0.3160	2.431	2.433
140	0.3380	2.414	2.415
150	0.3600	2.400	2.397
160	0.3810	2.381	2.378
170	0.4020	2.365	2.360
180	0.4230	2.350	2.341
190	0.4440	2.337	2.322
200	0.4640	2.320	2.304

It will be seen that the reducing powers of xylose and arabinose are almost identical. For practical purposes when working with the unknown pentoses in plant extracts it is probable that no large error will be incurred by taking as the divisor the average value for arabinose and xylose corresponding with the weight of CuO dealt with. The reducing powers of arabinose and xylose differ too only slightly from that of dextrose; thus the divisors for these three sugars for 100 mg. of sugar, are respectively 2.536, 2.490 and 2.538.

Pentoses are generally present in appreciable quantity in the solutions obtained by extracting foliage leaves with alcohol; when such solutions have been treated with basic lead acetate in the usual way and the excess of lead has been removed by means of sodium carbonate, or other precipitant, these pentoses exercise a reducing action on Fehling's solution. When, therefore, it is necessary to make an analysis of such material, allowance must be made for these sugars, as indicated in the scheme given on page 64, before it is possible to calculate the proportion of other reducing sugar present, such as dextrose and lævulose.[1]

Kluyver (*Biochemische Suikerbepalingen*, 1914, page 181), considered that pentoses in the free state do not usually occur in plant extracts but Davis and Sawyer[2] have since given definite proof of their presence in extracts of certain leaves (turnip, mangold). Kluyver emphasised the fact that when other sugars, such as cane sugar, lævulose, etc., are present, the values obtained by the ordinary Tollens-Kröber method of estimating pentoses by distillation with hydrochloric acid are high owing to the formation of a furfural-like substance (probably hydroxymethylfurfuraldehyde) which yields an insoluble phloroglucide; the presence of pentoses might therefore

[1] Davis and Daish. *J. Agric. Sci.*, 1914, **5**, 465.
[2] *J. Agric. Sci.*, 1914, **6**, 406.

be inferred in cases when these sugars were really absent, owing to the action of the hydrochloric acid used on sugars such as saccharose, dextrose and lævulose which were present in relative excess. Kluyver also suggested that the pentose estimation should only be carried out after fermenting away the other sugars. Davis and Sawyer, however, show (loc. cit.) that the error caused by the presence of these in estimating the pentoses in plant extracts is relatively small and for most practical purposes can be neglected.

Cunningham and Dorée[1] have discussed at some length the formation of hydroxyfurfuraldehyde from various carbohydrates. They show that the condensation which produces furfural in the case of pentoses or pentosans takes place rapidly and is almost completed before the hydroxymethyl-derivative begins to distil over. By using aniline acetate test paper it is possible to distinguish between the separation of the two aldehydes and practically no error is made in pentosan estimations if this indicator is used. The hydroxyaldehyde is produced at a very slow rate. Its total amount is small, varying between 1 and 2% in the case of the hexoses and those celluloses which contain little or no pentosan. The probable reason for this is the ready hydrolysis of the hydroxymethylfurfuraldehyde to formic and lævulic acids. This property explains the well-known observations of Fraps[2] who found that when the distillates obtained from a number of natural products, food stuffs, etc., were distilled again, a considerable loss of "furfural" occurred. With pure furfuraldehyde this did not occur. He considered that the portion which disappeared could not be regarded as due to true pentosan and in the absence of further knowledge designated it as the "furaloid" constituent. In all probability the "furaloid" is a hexose-yielding constituent which gives hydroxymethylaldehyde and this on a second distillation is largely decomposed.

The formation of the hydroxymethylaldehyde under the conditions given above, makes estimations of methylpentosans, by the method of Ellet and Tollens[3] of doubtful value.

Estimation of Carbohydrates in Plant Extracts.—The following scheme for the analysis of plant material such as foliage leaves, seeds, etc., has been suggested by Davis and Daish.[4]

The material immediately, after picking, is dropped into a large volume (2 litres) of boiling alcohol to which a little ammonia has been added (10 to 20 c.c. of ammonia, sp. gr. 0.880). This treatment destroys all the enzymes which are present and prevents change of the sugars during subsequent treatment.

The plant material is extracted in a large metal Soxhlet extractor for 18 hours. The extract is then evaporated in vacuo (60 to 20 mm.) to a small bulk and made up to a definite volume, e.g., 500 c.c. Of this, two portions

[1] Biochem. J., 1914, 8, 438.
[2] Amer. Chem. J., 1901, 25, 201.
[3] J. Landwirth, 1905, 53, 13.
[4] J. Agric. Sci., 1913, 5, 437.

of 20 c.c. each are evaporated to dryness and dried *in vacuo* for 18 hours at 100° C. This gives the total dry matter in the extract. 440 c.c. are treated with the requisite volume of basic lead acetate solution, filtered under pressure on a Buchner funnel, washed and made up to a known volume, 2 litres. This is called Solution A.[1]

300 c.c. of Solution A are deleaded by means of solid Na_2CO_3, avoiding much excess, and made up to 500 c.c. This is called Solution B.

(1) 25 c.c. of B are used for the *direct reduction* and *polarisation;*[2] the reduction is due to dextrose, lævulose, maltose, pentoses.

(2) **For Saccharose.**—Invert 50 c.c. of B:

(*a*) By invertase. Make neutral to methyl-orange by a few drops of concentrated sulphuric acid, and add 1–2 c.c. of autolysed yeast and 2 or 3 drops of toluene and leave 24 hours at 38–40° C. After this period, add 5 to 10 c.c. alumina cream, filter and wash to 100 c.c. Take the reducing power of 50 c.c. (=25 c.c. B) and polarise.

(*b*) By 10% citric acid. Make faintly acid to methyl-orange by adding a few drops of concentrated sulphuric acid and add a weighed quantity of citric acid crystals so as to have 10% of the crystalline acid ($C_6H_8O_7+H_2O$) present. Boil 10 minutes, cool, neutralise (to phenolphthaleïn) with sodium hydroxide, make to 100 c.c. and determine the reducing power of 50 c.c. (= 25 c.c. B); polarise in faintly acid solution.

Saccharose is calculated from the increase of reducing power or change of rotation caused by inversion. The values obtained by the two methods (*a*) and (*b*) should agree closely.

(3) **For Maltose.**—Another 300 c.c. of Solution A is deleaded by means of hydrogen sulphide and filtered, the precipitated sulphide being washed until the total volume of filtrate and washings is about 450 c.c. Air is then sucked through this for about 1½ hours to expel hydrogen sulphide, a very little ferric hydroxide is added to remove the last traces of the latter, and the solution is made to 500 c.c. It is filtered and

50 c.c. fermented (*a*) with S. marzianus
50 c.c. fermented (*b*) with S. anomalus
50 c.c. fermented (*c*) with S. exiguus

and two lots *d* and *e* of 50 c.c. are fermented with baker's yeast. It is generally necessary in order to ensure good growth of the yeast to reduce the acidity by adding 2 to 5 c.c. of *N*-sodium carbonate to the 50 c.c. to be fermented; 5 c.c. of sterilised yeast water is also added, the mixture is sterilised in the usual way and inoculated in the inoculating chamber with the pure culture

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[1] It is often preferable when the solutions contain relatively small quantities of sugars to add sodium carbonate (or sodium-sulphate) to remove the excess of lead before completing the volume to 2 litres. In such cases solution A, after filtering, is used directly for the sugar estimations.

[2] The polarisation of these dilute solutions is usually small and it is therefore necessary to take the reading in a long tube (at least 200 mm. in length) with an instrument reading accurately to ¹⁄₁₀₀°, the temperature being maintained constant at 20° C. within ¹⁄₁₀°. It is an easy matter, using a Lowry thermo-regulator and circulating the water by means of a small pump, to keep the temperature constant to ¹⁄₁₀₀°, but differences of temperature less than ¹⁄₁₀° hardly make a perceptible difference in the readings with such dilute solutions as these.

If (as is usually the case) amides and amic acids are present, which have an optical rotatory power, it is advisable to make the solution acid before measuring the rotatory power; for this purpose sulphur dioxide should be passed through the solution, as suggested by Pellet (see p. 44).

of yeast. It is then stoppered with cotton wool and the yeast allowed to incubate for 21 to 28 days at 25°.

After completion of the fermentation, 5 c.c. of alumina cream are added, the solution made up to 100 c.c. at 15°, filtered and 50 c.c. are used for the reduction. The difference between the average reduction with *a*, *b* and *c* and the average of *d* and *e* gives the reduction due to *maltose*.

(4) **Pentoses.**—These are approximately determined in 50 c.c. of A by distilling with hydrochloric acid according to the Tollens-Kröber method (see p. 63).

(5) When the reduction in (1) due to pentose and maltose has been allowed for, using the values for the reducing power of the pentoses given by Daish (see p. 62), the remaining reducing power is due to dextrose and lævulose; the actual proportions of these sugars, *in the absence of other substances possessing a rotatory effect on polarised light*,[1] can be calculated from the reducing power of the solution, combined with its corrected specific rotatory power, as suggested by Brown and Morris.[2]

A simple apparatus for evaporating plant and animal extracts *in vacuo*, such as is necessary in the above scheme of analysis, has been described by the writer. By means of this apparatus all the difficulty encountered with such material, owing to the tendency to frothing which usually occurs, is completely overcome. Large volumes of liquid can be evaporated continuously and the distillate recovered, if necessary in fractions; the apparatus requires practically no watching after the distillation has once been started and can be left to itself whilst other work is proceeded with. It is only necessary from time to time to renew the liquid in the distilling flask A, by means of the dropping funnel A′.

The apparatus consists of an ordinary distilling flask with the side-tube bent up and passing into a wide piece of glass tube B which serves as a froth-trap; the latter is connected by glass tubing with the condenser D, the lower end of which passes through a rubber stopper into the cylindrical dropping funnel E, which in turn is connected, as shown, below with the pump flask G and above with the large reservoir P, which serves to take up small variations of pressure and thus ensure a steady vacuum throughout the system. In this way regular ebullition, without overheating or frothing, is secured.

The vacuum is maintained by means of an ordinary water injector-pump, connected through a Hutchinson regulating valve *J* (*Chemical News*, 1912, 99) with the bottle *H* and thence with *E* and *G*; a glass cock is interposed at *T*, whilst *S* is a screw-clamp which operates on the piece of rubber pressure-tubing connecting *G* and *H*. At *M* a manometer tube is inserted which shows the vacuum throughout the system. The Hutchinson valve takes up large

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[1] Such substances are asparagine, glutamine, aspartic and glutamic acids, which occur widely in plant material and are not removed in the ordinary process of treatment by basic lead acetate, etc.; these substances are likely to cause error in the estimation of sugars in plant extracts and in materials such as beet-sugar molasses.
[2] *Trans.*, 1893, 63, 604.
[3] *J. Agric. Sci.*, 5, 434; *Chem. World*, 1914, 3, 239.

variations in the vacuum due to changes of water pressure, so that by means of this, combined with the regulating reservoir P, changes in the vacuum are reduced to a minimum.

When the liquid in A first begins to boil there is often a great tendency to froth; should this occur, the froth rises into the trap B, breaks against a disc of copper-gauze, and the liquid is returned automatically to the flask through the piece of glass tube L.

The combination E and G allows of the distillate being removed from time to time; whilst the distillation is proceeding, the vacuum in G is main-
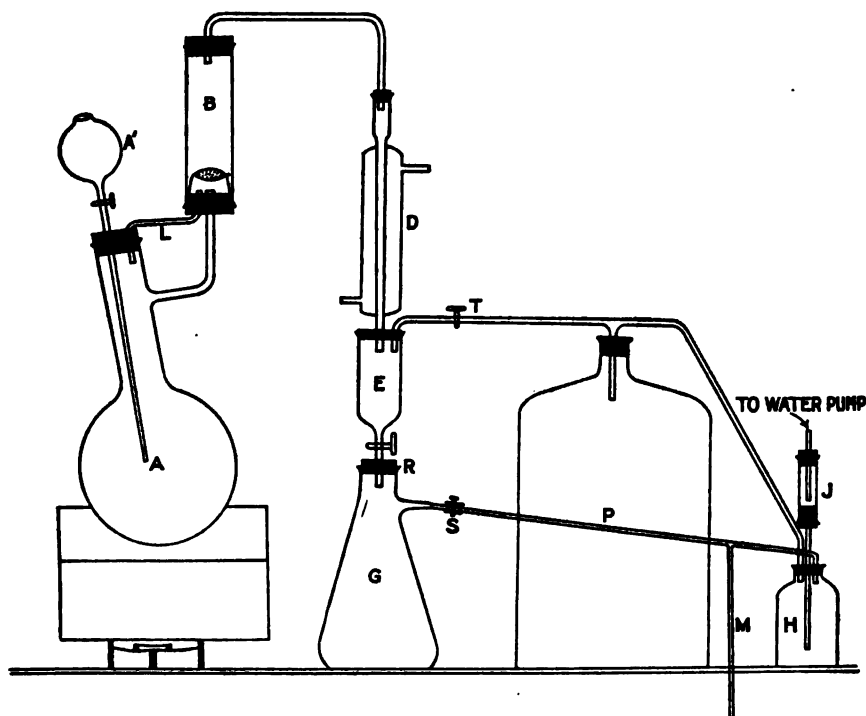
FIG. 2.

tained the same as in the rest of the system so that by opening the glass tap of E the distillate runs down into G. When G is full and it is required to empty it, the cock E is closed and the screw-clamp of S screwed down to the rubber pressure tube. The latter is then detached from the side tube of G and the flask G removed from the rubber stopper R, emptied and replaced without interfering with the vacuum throughout the rest of the system. After it has been replaced, S is opened and in a very short time the vacuum is reëstablished in G, the same as throughout the rest of the apparatus.

It is a simple matter by introducing T-pieces to run two or more of these distilling apparatus in conjunction with a single vacuum pump and a single

regulating vessel *P*. All connections must of course, be made with rubber stoppers or rubber pressure tubing.

ERRATA IN VOL. I.

Page 286, line 5 from bottom for "proteins" read "protein."

Page 290, line 10 for $W = \dfrac{1000\,W}{D}$ read $W = \dfrac{1000\,w}{D}$.

Page 290 line 16 for "water" read "solution."

Page 301 for "Levo" read "lævo" throughout.

Page 303, in the table, under "Milk Sugar "insert" anhydrous." In the milk sugar column for "67.8" read "78.4," for "55.8" read "55.3."

Under "maltose" for 139.0 read 138.0.

Page 306, in bottom table, insert after "Lactose" the word "hydrate." Insert also: Lactose, anhydrous $[\alpha]_D = +55.3$.

Page 311, line 3 from bottom the reference should be "Zeit. Ver. Deut. Zuckerind."

Page 311, line 12 "Diot," should read "Dist."

Page 314 line 16 from bottom for "sentianose" read "gentianose."

Page 318, line 12 for 175 read 173.

Page 319, line 4 from bottom for "0.0678 grm. lactose "read" 0.0784 lactose anhydrous."

Page 322, line 6, from bottom for "two" read "ten."

Page 323, line 2, for "detail is" read "details."

Page 328, the table at head of page 328 should be amended as follows:

	Glucose, $C_6H_{12}O_6$	Cane sugar, $C_{12}H_{22}O_{11}$ (after inversion)	Lactose, $C_{12}H_{22}O_{11} + H_2O$	Lactose (anhyd.), $C_{12}H_{22}O_{11}$	Maltose, $C_{12}H_{22}O_{11}$
Copper, Cu...........	0.5676	0.5392	0.7621	0.7240	0.9155
Cuprous oxide, Cu₂O..	0.5042	0.4790	0.6769	0.6431	0.8132
Cupric oxide, CuO.....	0.4535	0.4308	0.6088	0.5784	0.7314

As the reducing power of the sugars varies with the amount of sugar present, these tables are only strictly correct for one concentration of the sugar solution. It is therefore more exact to make use of the table of Brown, Morris and Millar (page 27) which gives the reducing power for different quantities of cupric oxide precipitated.

Page 328, line 9 from bottom, transpose "by" and "of."

Page 329, line 9 from "bottom for "formulas" read "formulæ."

Page 330, for "y" read "Y" throughout.

Page 331, bottom-line delete %.

Page 332, line 20 from bottom for "solution" read "solutions."

Page 335, line 11 for "cabonate" read "carbonate."

Page 337, line 8 from bottom, for "has" read "have."

Page 340, line 12 from bottom delete "with."

Page 342, line 20, "matters" should be transferred to 4 lines higher up, before "should," "really" should read "readily."

Page 344, line 6, for "in" read "is."

Page 358, line 12 from bottom for "eight" read "seven."

Page 362, line 17 from bottom, the later value for K (absolute) of maltose (Brown, Morris and Millar *Trans.*, 1897, 100) is 62.2.

Page 363, line 2 for "reaction" read "solution."

Page 365, the value for lactose $[\alpha]_D = 52.7$ should be $52.5°$ and refers to hydrated lactose, $C_{12}H_{22}O_{11},H_2O$. For anhydrous lactose, $[\alpha]_D^{20°} = 55.3$. In line 14 from bottom the reducing power of anhydrous lactose is roughly $\frac{2}{3}$ that of dextrose.

Page 375, line 13 from bottom, read "invert sugar is usually a syrup."

Page 376, line 15 from "caclulated" read "calculated;" line 8 from bottom for "percentum" read "percentage."

Page 387, line 19, delete "degrees."

Page 401, line 8, for "læonlose" read "lævulose."

Page 401, line 11 from bottom, for "anhydrid" read "anhydride."

STARCH AND ITS ISOMERIDES.

BY WILLIAM A. DAVIS.

A study of the methods of estimating starch has recently been published by Davis and Daish.[1] A new method, based on the use of taka-diastase, has been proposed, which is at once more accurate and more generally applicable than any of the processes which have yet been suggested. The following summary of the paper may here be given.

The modified Sachsse method (Vol I, p. 420), which is Official in the United States of America[2] and is based on the hydrolysis of the starch present with boiling dilute acid, is valueless in the majority of cases when dealing with plant material, because of the presence of pentosans, "hemi-celluloses" and other substances which yield reducing sugars that count as dextrose; even when such substances are absent the method gives results which are more than 5 per cent. low, owing to the destruction of dextrose that occurs during the prolonged treatment with acid which is a feature of this method.[3] In a series of analyses made by this process, of samples of purified potato starch dried *in vacuo* at 120°, results ranging from 93.8 to 94.3% of starch were found, whereas by the ordinary O'Sullivan diastase process an average result of 100.1% was obtained; with taka-diastase (see p. 72) the average result with the same sample was 99.6%, a value which probably represents the real starch more closely than the figure obtained by the ordinary diastase process, the calculations of which depend upon a value for the specific rotatory power of dextrin derived from observations made with similar material. Carefully purified starch always contains small quantities of protein material, cellulosic tissue and ash, so that the figure 99.6% obtained with the taka-diastase is probably a close approximation to the percentage of true starch, and the acid process shows a loss of more than 5%. The destruction of dextrose is a source of error in all the methods which make use of hydrochloric acid to effect hydrolysis, such as that of Märcker and Morgen, even when the primary conversion of the starch has been carried out with diastase.

Although ordinary diastase gives with purified starch results by O'Sullivan's method which are approximately correct, values 15 to 20% lower than the actual starch content may be obtained when it is applied to leaf material or plant tissues in general, owing to the loss of dextrin. In the majority of cases, plant material, which has been previously deprived of sugar by prolonged ex-

[1] *J. Agric. Sci.*, 1914, **6**, 152.
[2] *Bureau of Chemistry*, Bulletin 107, Revised, 1912, page 53.
[3] Compare *J. Agric. Sci.*, 1913, **5**, 437.

traction with alcohol, still contains tannins, amino-acids, proteins, etc.; during the hydrolysis by diastase these pass into solution and exercise a very marked effect on the reducing power and optical activity of the solution. These substances have therefore to be removed by the addition of basic lead acetate, which almost invariably produces a heavy precipitate in the filtered solution obtained from the diastase conversion. Although basic lead acetate *does not of itself precipitate dextrin*, when dextrin is present in solutions in which a precipitate is produced; as in the purification of the solutions obtained from the diastase conversions, it is *carried down with this precipitate and is thus lost to the analysis*.[1]

Taka-diastase as an Agent in Estimating Starch

To estimate starch in foliage leaves and in similar cases in which it is necessary to purify the solution after hydrolysis has been effected, it appeared probable that the so-called "taka-diastase" would be more suitable than ordinary diastase, as it is said to give rise only to maltose and dextrose (compare Croft-Hill, *Proc. Chem. Soc.*, 1901, **240**, 184) free from dextrin.[2] If this were the case, it would be possible to add basic lead acetate or other clarifying agents without losing sugars. The results given in the following table show that this is actually true and that the use of taka-diastase affords an accurate means of estimating starch in cases where the ordinary diastase process is quite unsuitable. The product of the action of taka-diastase on starch consists, after the first three hours, solely of a mixture of maltose and dextrose; as time proceeds the amount of dextrose increases at the expense of the maltose, which is gradually converted by the enzyme maltase present in the taka-diastase, into two molecules of dextrose. The curve given (Fig. 3) shows this. The dextrose curve represents the dextrose formed from 100 grm. of starch, *calculated as starch* (by multiplying the dextrose figure at each period by 0.9), the maltose curve similarly showing the maltose as starch (dividing the maltose figure by 1.055). This system of plotting the results shows for every instant the proportion of the *original starch which is present either as maltose or dextrose*, the sum of the maltose and dextrose values at each point being approximately 100, so that the two curves are complementary.

From the shape of the curves, coupled with the results found after 3 hours, it is probable that the first action of the taka-diastase is to break down the starch to dextrin and maltose, just as in the case of ordinary

[1] When basic lead acetate is added to the solution obtained by the diastase conversion of *purified starch* not the slightest precipitate is produced with the dextrin existing in solution; but results obtained in a series of special experiments showed that if sodium carbonate is subsequently added, or hydrogen sulphide is passed so as to precipitate the lead, a greater or smaller proportion of the dextrin is removed by co-precipitation.

[2] In 1898 Stone and Wright (*J.Amer. Chem. Soc.*, **20**, 639–647) attempted to estimate starch by means of taka-diastase; but as they assumed maltose to be the only sugar formed and measured the products of the action solely by the reducing power without reference to their rotation, they concluded that under their conditions "taka-diastase is not adapted for use in the quantitative estimation of starch."

TABLE I.—ACTION OF 0.1 GRM. TAKA-DIASTASE ON POTATO STARCH AT 38°.

Time in hours	Weight of starch dried in vacuo at 120°	CuO from 25 c.c. of 500 c.c.	a_D in 200 mm. tube at 20.00°	Dextrose in 500 c.c.	Maltose in 500 c.c.	Total starch	% starch found	Dextrose/Maltose	Remarks
3	2.0061	0.1321	1.192°	....	....	....	......	....	Dextrin still present. No alumina cream added.
	1.9978	0.1380	1.273	....	....	....	......	....	
6	2.0099	0.1581	1.103	0.2204	1.892	1.9914	99.55	0.116	No alumina cream.
	1.9914	0.1535	1.097	0.1640	1.932	1.9776	99.34	0.085	No alumina cream.
12	1.9752	0.1780	0.991	0.5328	1.5930	1.9884	100.7	0.334	No alumina cream.
	1.9940	0.1800	0.973	0.5748	1.5430	1.9792	99.37	0.372	5 c.c. alumina cream.
24	2.0148	0.2189	0.808	1.1596	1.0214	2.0112	99.80	1.135	5 c.c. alumina cream.
	1.9876	0.2205	0.764	1.2302	0.9148	1.9739	99.33	1.345	5 c.c. alumina cream.
	2.0145	0.2218	0.783	1.2204	0.9534	2.0018	99.37	1.280	No alumina cream.
	2.0046	0.2212	0.777	1.2218	0.9414	1.9916	99.39	1.298	5 c.c. alumina cream.
	2.0024	0.2167	0.794	1.1546	0.9982	1.9846	99.11	1.157	5 c.c. basic lead added and this pptd. by solid Na₂CO₃.
48	2.0000	0.2561	0.594	1.8106	0.3852	1.9942	99.71	4.700	No alumina cream.
	2.0000	0.2544	0.616	1.7674	0.4416	2.0086	100.40	4.002	5 c.c. alumina cream.
72	2.0016	0.2588	0.556	1.886	0.2874	1.9703	98.45	6.563	5 c.c. alumina cream.
	1.9961	0.2562	0.562	1.852	0.3113	1.9620	98.28	5.950	5 c.c. alumina cream.
	2.0084	0.2571	0.569	1.8536	0.3231	1.9741	98.33	5.737	5 c.c. alumina cream.
	2.0046	0.2568	0.566	1.8538	0.3178	1.9696	98.27	5.833	5 c.c. alumina cream.
96	2.0000	0.2592	0.562	1.8838	0.2992	1.9790	98.95	6.296	5 c.c. alumina cream.
	2.0048	0.2590	0.572	1.8694	0.3226	1.9879	99.15	5.794	Nothing added.
	2.0054	0.2569	0.562	1.8600	0.3082	1.9660	98.05	6.034	5 c.c. alumina cream.
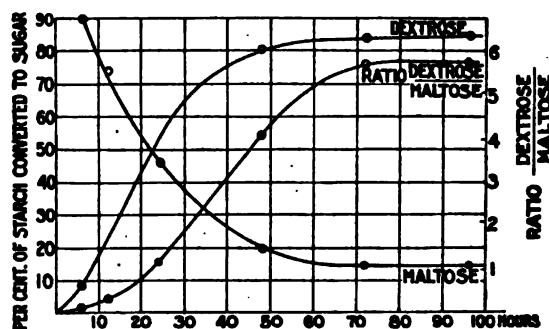
FIG. 3.

diastase; the maltase comes into action comparatively slowly, so that after 6 hours only $\frac{1}{10}$ of the original starch is present as dextrose. Subsequently, however, the rate at which dextrose is formed increases, following very nearly a straight line curve between 6 hours and 28 hours, when about 60% of the starch is present as dextrose; the rate of formation of dextrose then rapidly slows down until a nearly constant value is reached in the neighbourhood of 84%.

The view that has recently been put forward by Kita,[1] that when taka-diastase acts on starch the dextrose is split off directly from the starch and is

[1] *J. Ind. Eng. Chem.*, 1913, 5, 220.

therefore not formed through the intermediary of maltose, is highly improbable in view of the above results.[1] Davis and Daish give analyses made with maltase-free yeasts confirming the view based on these results, that the actual product of the conversion throughout is a mixture of maltose and dextrose only; after 6 hours every trace of dextrin has disappeared under the conditions employed.

At 55°, the optimum temperature for the action of ordinary diastase, the action of taka-diastase, at least as regards the transformation of maltose into dextrose, is very much restricted, owing to the fact that the enzyme maltase is gradually destroyed at the higher temperature. In working with taka-diastase, therefore, a *temperature of 38 to 40° should not be exceeded.* It is noteworthy that the average value for the starch present in the purified potato starch found on using taka-diastase in the conversions of 6 to 48 hours is 99.65, whereas with ordinary diastase a value generally 0.5 per cent. higher was obtained (see page 70). It is probable that the numbers obtained with taka-diastase more nearly represent the true starch values, as the calculations are based on the constants for two pure sugars only; they do not involve any assumption with regard to specific rotatory power for the "dextrin" existing in solution, which is generally taken at 202°, although some doubt may still be entertained as to the exactness of this value. It is probable too that the purified potato starch contains a small proportion of foreign material; hence the low value of 99.6%.

When the conversions with taka-diastase are prolonged beyond 48 hours, somewhat lower starch values are generally obtained, as is seen in Table I; it is possible that some slight destruction of the sugars may occur during these prolonged conversions, but the lower values may also be due to the fact that a larger proportional error is incurred in reading the rotation. In Table I, the actual readings in a tube of 200 mm. for the longer conversions range only from about 0.5 to 0.6°; an error of 0.005° in the reading would therefore represent an error of 1%.

The use of taka-diastase in starch estimations has the advantage that it gives rise to two *sugars*, maltose and dextrose, the rotatory powers of which have been carefully determined; the temperature coefficients for these are exceedingly small so that no very special precautions to ensure exact constancy of temperature are necessary in ordinary work.

In actual analytical practice it is an easy matter to arrange the quantities so that considerably higher rotations are observed and the proportional error in this direction diminished; if possible, a 400 or 600 mm. tube should be employed. That the addition of precipitating agents such as alumina cream and basic lead acetate and the formation of heavy precipitates, such as are produced with lead by hydrogen sulphide and sodium carbonate, do not in the least influence the results, is shown by the analyses in Table I and numerous other experiments which need not be described.[2]

[1] See Davis, *J. Soc. D. and Col.*, 1914, 7, 249.
[2] Revis and Burnett (*Analyst*, 1915, October) have successfully applied taka-diastase to the estimation of starch in cocoa, which hitherto presented special difficulties.

Estimation of Starch by Means of Taka-diastase.

To estimate starch, the dry material (free from sugars[1] and, if necessary, previously extracted with water to remove gums, amylans, etc., see page 75) is gelatinised with 200 c.c. of water in a 250 c.c. beaker flask heated for $\frac{1}{2}$ hour in a water-bath at 100°. The solution is cooled to 38°, 0.1 grm. taka-diastase added,[2] together with 2c.c. of toluene and the mixture left 24 hours in order that the conversion may take place; it is then heated in a boiling water-bath to destroy the diastase and the clear solution above the residual material is filtered through a fluted filter paper into a 500 c.c. measuring flask; the residue is thoroughly washed several times by decantation, the washings being passed through the filter paper until the volume of liquid in the flask amounts to about 475 c.c. The necessary quantity of basic lead acetate is then added to precipitate the tannins, etc., present in the solution; the amount required varies considerably with different leaves, generally ranging from 5 c.c. to 25 c.c. A large excess of lead should be avoided and tests should be made after each small addition of lead acetate in order to ascertain when the precipitation is complete. When this is the case the solution is made up to 500 c.c. at 15°, and filtered; 100 c.c. of the filtrate are placed in a 110 c.c. measuring flask, the slight excess of lead precipitated by adding solid sodium carbonate and the volume adjusted to 110 c.c. at 15°. 50 c.c. of the filtrate from the lead carbonate are used for the reduction and another portion polarised in a 400 mm. tube. The following example shows the method of calculation:

Weight of extracted leaf material (*Tropæolum majus*) after drying in steam oven = 10.4122 grm.
Weight of leaf material dried *in vacuo* at 100°............................... = 9.4059
CuO from 50 c.c. of the final 110 c.c.. = 0.4492 grm.
Polarisation of this solution in 400 mm. tube at 20.00°...:.............. = 1.995°
         If x = grm. dextrose in 50 c.c. of this solution.
         y = grm. maltose in 50 c.c. of this solution.

we have, using the values of CuO corresponding to 1 grm. of dextrose and maltose for the weight 0.4492 CuO in the tables of Brown, Morris and Millar:

$$2.369x + 1.362y = 0.4492 \tag{1}$$

For the 400 mm. tube, employing the values $[\alpha]_D^{20°} = 137.6$ and $[\alpha]_D^{20°} = 52.7$ for maltose and dextrose we have also

$$4.216x + 11.008y = 1.995° \tag{2}$$

Solving equations 1 and 2 for x and y
         x = 0.1095 grm. dextrose in 50 c.c.
         y = 0.1394 grm. maltose in 50 c.c.

Total dextrose in 500 c.c. original solution = 0.1095 $\times \dfrac{110}{50} \times \dfrac{500}{100}$ = 1.2045 grm

Total maltose in 500 c.c. original solution = 0.1394 $\times \dfrac{110}{50} \times \dfrac{500}{100}$ = 1.5334 grm

Starch corresponding to dextrose = 0.90 $\times$ 1.2045 = 1.0840 grm.
Starch corresponding to maltose = 1.5334 + 1.055 = 1.4535 grm.
         Total starch = 2.5375 grm.
∴ % of starch in vacuum dried extracted leaf material
$$2.5375 \times \frac{100}{9.4059} = 26.97\%.$$

[1] To remove sugars the material should be thoroughly extracted by boiling 80 % alcohol in a Soxhlet extractor. For the details in the case of leaf material and other vegetable tissue, see Davis and Daish (*loc. cit.*); precautions should be taken in such cases to ensure that all enzymes present are destroyed at the moment the sample is taken.
[2] We have used the commercial preparation of Messrs. Parke, Davis & Co.

Precautions Necessary in Taking Samples for Analysis.

If the dried, ground plant material is bottled before analysis, it is absolutely necessary when each sample is taken for the analysis, *to turn out the whole of the material on to a sheet of paper and thoroughly mix it before sampling.* If this precaution is not observed and successive samples are taken directly from the bottle, it is frequently found that the proportion of starch present in the material increases toward the bottom of the bottle. This is no doubt due to the fact that the heavier starch grains, set free from the tissue by grinding, sink to the bottom of the bottle, whilst the lighter fibrous material rises to the top. This is well shown by the following successive analyses made with potato leaves (previously freed from sugars by extraction):

    1 sample from top of bottle, starch = 7.54% on vacuum dried matter.
    2 sample from middle of bottle   = 9.19% on vacuum dried matter.
    3 sample from middle of bottle   = 9.23% on vacuum dried matter.
    4 sample from bottom of bottle   = 12.29% on vacuum dried matter.

When, however, the sampling is carried out in the way described above the agreement between different individual determinations is as satisfactory as could be expected in this class of work.

One of the principal difficulties in estimating starch in plant material is due to the presence of gummy substances, tannins, proteins, etc., which pass into solution during the hydrolysis and exercise an effect on the rotatory and reducing power of the solution. These substances are very largely removed by the use of basic lead acetate, but sufficient impurity remains, even after this treatment, to falsify the analyses in some cases. Thus in the case of mangold leaf a lævorotatory gum is present, which gives an error of nearly 10% on the rotation actually measured.

In working with plant material it is generally possible to extract the disturbing gummy substance prior to the starch conversion by a preliminary treatment with water.[1] Thus in the case of the mangold leaf, by adding 200 c.c. of water and 5 c.c. of toluene to the leaf material and extracting for 24 hours at 38°, decanting and washing with a little water and subsequently converting with taka-diastase, in the ordinary way, a solution is finally obtained (after the usual treatment with basic lead and sodium carbonate) which in a 400 mm. tube shows a lævorotation of not more than 0.01°. It is noteworthy that the preliminary treatment with water fails to remove the greater part of the material precipitable by basic lead acetate, so that this treatment is necessary even after the preliminary extraction with water.

In the case of plant material from which gummy matter is extracted with extreme difficulty, it would probably be sufficient to introduce a correction for any active substances present by carrying out a control experiment or "blank" in which the diastase is omitted but the material is otherwise treated exactly as in the actual estimation of starch.

[1] Compare Brown and Millar, *Trans. Guinness Lab.,* Vol. I, 79.

Ewer's Method of Estimating Starch in Cereals.

This is a method which has come into considerable use especially in dealing with brewers' materials (barley, grains, etc.). It is a modification of Lintner's polarimetric method (see Vol. I, page 424) based on the substitution of hot dilute hydrochloric acid for the cold acid used in this process. The following is a description of the process as applied to barley.[1]

The barley is coarsely triturated, and then ground in a Dreef mill to such a degree that the meal passes through a half-millimetre sieve (with the exception of a small quantity of husk which is subsequently mixed with the sifted portion). 5 grm. of the grist are mixed and shaken in a 100 c.c. flask with 25 c.c. of dilute hydrochloric acid (containing 1.124% of hydrochloric acid by weight), the neck of the flask being rinsed down with a further quantity of 25 c.c. of the same acid. The flask is rotated several times and then placed in a boiling water-bath for exactly 15 minutes during the first three of which it is repeatedly rotated. The solution is then made up to about 90 c.c. with cold water, cooled to 20°C., clarified with 2 c.c. of sodium molybdate solution (prepared by fusing 30 grams of pure molybdic acid and 25 grams of pure, dry sodium carbonate, dissolving the product in water to 250 c.c. and filtering) or 10 c.c. of 4% phosphotungstic acid solution, made up to 100 c.c. with water, mixed, filtered and polarised. The solution, after polarisation, may be treated with a few drops of molybdate solution to make sure that all the protein has been thrown down. The starch solutions are very stable, and show no decrease of rotation after standing for 24 hours. The author finds that the specific rotation of barley starch is $[\alpha]_D^{20°} = 181.5°$. With a half-shadow saccharimeter (Ventzke-Soleil or German sugar scale, and Auer light with a light filter, the percentage of starch is obtained by multiplying the reading of a 5% solution in a 200 mm. tube by 1.912; the specific rotation of the starch solution is independent of the concentration between the limits of about 1.25–5%. The above method is preferable to those of Lintner and Wenglein in that the use of concentrated acids is avoided; moreover, there is no need to grind the meal very finely. On the other hand, in Lintner's method, heating and cooling the solution are avoided.

For wheat and other cereals the following specific rotations are used for the starch:

Wheat	182.7	Rye	184.0
Barley	181.5	Oats	181.3
Rice	185.9	Maize	184.5
Potato	195.4		

Whilst the method may be useful in certain cases for giving comparative results, it is very doubtful whether the values obtained represent the *true starch* present, owing to the fact that the treatment with hydrochloric acid breaks down pentosans and hemicelluloses, substances with a dextrorotation

[1] *Zeits. ges. Brauw.*, 1908, **31**, 250.

passing into solution which count as starch; thus the writer found that a sample of mangold leaf, which was absolutely free from starch, when subjected to Ewer's method gave a solution having a strong dextrorotatory power, which corresponded with the apparent presence of 16.5% of starch. Far higher values, too, are obtained by this method for the starch present in grain, such as wheat, than are given by the diastase method, so that it must be regarded as a process the reliability of which is very much open to question.

For the theory of the action of enzymes on starch see Davis (*J. Soc. D. and Col.*, 1914, **7**, 249); of the action of acids on starch, *ibid.* and Daish, (*Trans.*, 1914, **105**, 2053 and 2065).

Cellulose.

König and Hühn[1] have carried out a comparative series of estimations of cellulose in typical raw materials by the principal methods which have been proposed and adopted by different workers. The materials tested included beech, oak and pine woods, the bark of these woods, sulphite wood pulp, cotton, flax, hemp and jute. The methods employed were those of Weende-Henneberg ("crude fibre"); of Tollens-Dmochowski (in which hydrolysis according to the Weende-Henneberg method is followed by oxidation with nitric acid); of König (see Vol. I, page 437) using ammoniacal hydrogen peroxide; of Cross and Bevan (chlorination); Schulze (potassium chlorate and nitric acid) and Müller (bromine water and ammonia). Of these methods that of Weende-Henneberg and the direct treatment with ammoniacal hydrogen peroxide give products which cannot be described as cellulose, being still rich in lignin, whilst others yield celluloses more or less profoundly affected by the oxidising agents employed and still containing appreciable residues of the non-cellulose constituents of the raw material. Consequently, the authors made investigations of the nature of the "cellulose" products obtained, not only by qualitative tests, which are of little real value, but by quantitative determinations of elementary composition, calorific value, methyl value, and yield of furfural (pentosans). Comparison of the values so obtained with those given by the original material throw light on the extent to which non-cellulose groups have been removed. Considerations of such data raise the question of a definition of cellulose. According to König and Hühn the original material contains "true cellulose," lignin, hemihexosans and pentosans, together with cutin in the case of bark tissue. They contend that only by a regulated hydrolysis followed by oxidation can the "true cellulose" be freed from the other constituents. Methods depending upon oxidation without hydrolysis (for example, Cross and Bevan's chlorination method) may under favourable conditions eliminate the greater part of the lignin, but such methods as a rule fail to remove most of the hemihexosans and

[1] *Zeit. Farb. Ind.*, 1911, **11**, 297, 326, 344, 366; 1912, **12**, 4, 17, 37, 57, 77 and 102.

pentosans and the higher yields so obtained depend upon the presence of these impurities; moreover, the cellulose obtained has pronounced oxycellulosic properties. For instance, Cross and Bevan's method yielded 100% of cellulose from cotton, whilst König's method gave only 88.27% in the form of a friable powder. Hence it is contended, cotton is not substantially composed of true cellulose but contains considerable proportions of hemihexosans.

König and Hühn suggest the following method of testing the purity of the preparations of cellulose: The cellulose is dissolved in a solution of zinc chloride in concentrated hydrochloric acid and the liquid examined polarimetrically at intervals over a considerable period, the specific rotatory power being calculated. This gradually rises from zero as hydrolysis proceeds, reaching a maximum after 2 or 3 days, then falling slowly, owing to condensation until the liquid becomes opaque. Cotton cellulose prepared by König's method showed a maximum $[\alpha]_D = 82.5°$ after 72 hours whilst cotton purified by Cross and Bevan's method showed a maximum of only 56.7° after 55 hours.[1] Hydrocellulose and oxycellulose reach only low maximum rotations and the rotation changes rapidly. König and Hühn consider that this supports the view that digestion with glycerin containing 2 grm. of sulphuric acid per 100 c.c. at a temperature of 137° whilst eliminating the hexosans and pentosans has practically no effect on the "true cellulose" and that König's method in spite of the low yields of cellulose obtained is the best method of estimating that constituent. Next to this method that of Tollens-Dmochowski is preferred; the hydrolysis of the hexosans and pentosans is, however, not complete.

Cross and Bevan[2] have pointed out that the chlorination process when properly controlled shows a minimum of secondary oxidising action being confined to a specific chlorination of the lignin groups. The "crude fibre" methods favoured by König and Hühn yield residual products of degradation by treatments more or less arbitrary because they are relatively non-selective in their actions, which are ill-defined through the complex and unascertained relation of the products to the parent substance. The manipulation, moreover, is too lengthy for technical purposes. Cross and Bevan consider that the chlorination process will remain the standard method of estimating cellulose.

Processes for the estimation of cellulose are necessarily at the present time of a purely conventional character; and for any special purpose in the textile industries a method which corresponds as closely as possible with the condi-

[1] It is doubtful whether any value can be attached to observations of this kind, in view of the extraordinary variations in the specific rotatory power of dextrose (one of the products of hydrolysis in such experiments) with changes of concentration of the hydrochloric acid and difference of temperature. As the question is essentially one of velocity of action, results of even comparative value can only be obtained if such factors as temperature, concentration, etc., are maintained rigidly constant. The extraordinary differences which are found in the rate of destruction of dextrose by hydrochloric acid of different concentration, as well as in the synthetic action of the acid on the sugar are also factors militating against its use for such comparisons (cf. Daish, *Trans.*, 1914, 105, 2053).

The physical condition of the material will also largely determine the relative rate of formation of cellobiose, dextrose and its decomposition products, upon which the maximum reached depends.

[2] 8th Intl. Congress App. Chem., 1912, Sect. VI A, 13, 101; *Zeit. Farb. Ind.*, 1912, 11, 197.

tions and yields of actual practice will probably be preferred. In such cases, "true cellulose" is still a substance to be defined. For the majority of purposes, there is little doubt that the chlorination process gives the most useful and most easily comparable data.

ERRATA IN VOL. I.

Page 406. The value $-24°$ given for $[\alpha]_D$ of α-amylan is really that of $[\alpha]_j$. The latest value of $[\alpha]_D$ is $21.6°$ (Brown and Millar, *Trans. Guinness Research Lab.*, p. 313). The value given for $[\alpha]_D$ of β-amylan is also that of $[\alpha]_j$; Brown and Millar give $[\alpha]_D = -129.7°$.

Page 410, line 5 from bottom for "comparision" read "comparison."

Page 419, line 15 from bottom, delete "a" at end of line.

Page 423, line 15 from bottom insert semicolon after "boiled."

Page 434, bottom line, for "hyoxide" read "hydroxide."

Page 435, line 15 from bottom delete "sub."

Page 445, line 2, delete "appl."

Pages 446 and 447 should be interchanged. In table on page 447, right-hand bottom corner, "page 392" should read "page 435."

Page 449, line 5, "Keldahl" should read "Kjeldahl."

Page 459, line 7, for "Blythe" read "Blyth," line 6 from bottom, delete final "to."

PAPER AND PAPER-MAKING MATERIALS.

By RALPH H. McKEE.

Bursting Strength.—The Mullen's paper tester[1] has been much improved by substituting a lever movement for the milled screw formerly used in fastening the paper. This change almost totally eliminates the personal equation formerly evident in working with this instrument.

In testing thin papers with this instrument the better practice is to use a thickness of four sheets and report one-fourth of the "points" (pounds per square inch bursting strength) obtained as the strength of the paper.

Thickness.—In measuring thickness of ordinary and thin papers it is becoming customary to take one-fourth of the thickness shown by the micrometer when four sheets are measured. This takes account of the "grain" of the paper and gives a result more nearly according with the result obtained when the paper is put into reams or into book form.

Sizes and Weights.—American practice differs much from the English in regard to sizes and weights of flat papers. In Canada the English practice is given the preference. Standard American sizes in inches are as follows:

Flat letter	10×16	Medium	18×23
Small cap	13×16	Double small cap	16×26
Flat cap	14×17	Royal	19×24
Demy	16×21	Double cap	17×28
Folio	17×22	Super royal	20×28

In America the 500 sheet ream is most commonly used though the 480 sheet ream is still used to some extent. The English 516 sheet ream is never used. The American "standard ream" of 24 × 36 in. is customarily used as a basis in calculating costs and in giving orders to the manufacturing departments.

Sulphate and Sulphite Fibres.—It has lately been found that undercooked sulphate fibre makes extra strong papers ("kraft" papers) such as are wanted for wrapping and envelope papers, a field previously controlled by unbleached sulphite fibre and the more expensive manila fibre. It is thus often desirable to see if a paper is a true "kraft" or whether it is partly or wholly a sulphite product. The following efficient method of distinguishing these two fibres we owe to Schwalbe.

Disintegrate the paper by a rasp or grater and remove rosin by extracting with alcohol and ether. Dry a small amount of the pulp. Heat it at 60 to 80° with 15 c.c. of $N/20$ ferric chloride for 10 minutes and the

[1] Vol. I, Fig. 76, 4th ed.

pulp will settle to the bottom of the beaker. Remove and reject all pulp adhering to the sides of the vessel. Filter and wash the remainder with warm water until no iron shows on testing the washings. Put the drained fibres in a small beaker, add 10 c.c. of 1% sulphuric acid and then 6 to 8 drops of a 2% solution of potassium ferrocyanide. Heat 5 to 10 minutes at 60 to 80° to bring out the colour. Wash and estimate by examining under the microscope the relative proportions of each kind present, counting the differently coloured fibres. The fibres of sulphite (unbleached) product are coloured a deep blue and those of "kraft" sulphate paper a faint yellowish, greenish or bluish colour.

It may be mentioned, though not ordinarily of interest when working with "kraft" papers, that bleached sulphite paper shows mostly pale blue but some deep blue fibres, soda paper pale blue and bleached sulphate uncoloured or faintly blue fibres.

Wood-pulp.

Four distinct methods are employed in the manufacture of wood-pulp, namely, the mechanical or ground wood, the sulphite, the sulphate and the soda process. The varieties of wood which commonly serve as raw materials are spruce, hemlock, poplar, white fir, balsam, pine and beech. Spruce is the most important of the pulp woods. Nearly 50% of the supply is converted into mechanical wood-pulp; the remainder being "reduced" by the sulphite process. Of the hemlock over 90% is manufactured into sulphite pulp, the other 10% being divided between the mechanical and soda processes. The wood structure of poplar and of beech is such that these woods are readily "reduced" by alkalies, and they are generally converted into soda pulp. The sulphate mill uses any coniferous wood, preference being given to spruce. White fir, balsam, and pine, can be converted by any process and consequently are used by the nearest mill irrespective of its type.

The soda process for the manufacture of wood-pulp, the oldest of the chemical methods employed for isolating the pulp fibre, consists in the digestion of deciduous woods with a hot solution of sodium hydroxide. The wood chips should be uniform in size, averaging 1 in. in length. It is unnecessary to remove knots, rotten places, or sawdust, because of the solvent action of the alkali. The chemical action of hydrolysis of the intercellular material gives rise to many organic compounds chief among which are the sodium salts of acids of the acetic series. The hydroxide is prepared by treating a solution of sodium carbonate, leached from "black ash," with milk of lime. The loss in sodium salts during the process is replaced by the addition of commercial soda ash.

Sindall and Bacon[1] give the following table showing the equivalent weights of recovered ash and of lime calculated from their percentage purity.

[1] *Paper Makers Monthly Magazine*, Aug., 1914.

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EQUIVALENT WEIGHTS OF RECOVERED ASH.

% Na₂O	Tons	Cwt.	Qr.	Lb.
48	100	0	0	0
46	104	6	3	23
44	109	1	3	1
42	114	7	2	0
40	120	0	0	0
38	126	6	1	7
36	133	6	2	18

EQUIVALENT WEIGHTS OF CAUSTIC LIME.

% CaO	Tons	Cwt.	Qr.	Lb.
100	100	0	0	0
98	102	0	1	18
96	104	3	1	2
94	106	7	2	17
92	108	13	3	19
90	111	2	0	26

These tables show the necessity of a close control of the causticising since a slight change in purity involves a considerable change in weights used. For example, if the percentage of Na₂O in the recovered ash changes from 48 to 40% one-fifth more weight of recovered ash will be required for causticising. In American practice it is customary to estimate the amount of soda ash in the black ash from the sp. gr. of its aqueous solution, but the same relationship between purity and amount used obtains.

The chips and liquor (8 to 10° Bé. NaOH) are introduced into either a stationary or rotary digester, the manhole closed and steam introduced until the charge is brought up to the maximum cooking pressure. This value varies in different mills but an average would be 100 pounds steam pressure. The maximum cooking pressure is maintained during from 3 to 7 hours depending on the quality of pulp desired. By increasing the time of cooking the yield is decreased but the pulp is of better quality, easier bleached and freer from shives.

At the close of the cooking the digester pressure is reduced to 30 pounds and the charge "blown" into the "blow-pit." The "black liquor" is allowed to drain thoroughly and the crude pulp is transferred to bins provided with false bottoms, in which it is washed free from alkali.

By increasing the cooking pressure the yield of pulp is reduced but the total time of cooking is shortened so that one factor offsets the other. Increase in the concentration of the sodium hydroxide, within limits, decreases the yield but improves the quality of the pulp.

Analysis of Soda Ash.—A 10 grm. sample is dissolved in 100 c.c. of water and 10 c.c., equivalent to 1 grm., are withdrawn by a pipette and introduced into a vessel for titration. A few drops of methyl-red or methyl-orange are added and the solution titrated with N-hydrochloric or sulphuric acid. The number of c.c. of acid used multiplied by 3.1 gives directly the percentage of Na₂O. If it is desired to report as Na₂CO₃ the titration multiplied by 5.3 gives the percentage.

Analysis of "Black Liquor."—E. Sutermeister[1] recommends the following method of analysing the highly colored "black liquors." To 300 c.c. of water containing 15 c.c. barium chloride (40 per cent.) add 25 c.c. of black liquor. Titrate with normal acid determining the end point by removing a drop at intervals and allowing it to fall into a thin layer of a dilute solution of phenol-phthalein contained in a beaker. When the drop no longer produces a pink colour the action is considered complete and the number of c.c. of acid used multiplied by 0.04 gives the weight of hydroxide present.

A second test is made by evaporating 25 c.c. of the liquor to dryness and burning off the organic matter. A titration is then made with normal acid using methyl-orange as the indicator. The difference in c.c. of acid between the two titrations multiplied by 0.053 gives the sodium salts of the carbon acids calculated as Na_2CO_3.

Lime used for the manufacture of soda pulp should be nearly pure calcium oxide 1.5 per cent. of silica, iron and alumina and other impurities is the ordinary maximum allowed, nor should the lime be air slaked.

The sulphate process for the manufacture of chemical wood-pulp is similar in principle to the soda process, differing however in the fact that the loss of sodium salts during the process is replaced by the introduction of crude sodium sulphate "salt cake," instead of by sodium carbonate. The salt cake, in a granular form, is introduced into the calcining furnace, where the "black liquor" is being burned to "red ash." The sodium sulphate reacting with the carbonaceous material present is reduced to sodium sulphide. As a result of incomplete reduction the product is contaminated with a varying percentage of sodium sulphate.

This ash, a mixture of sodium sulphide, sulphate, silicate and carbonate is dissolved in water and causticised with lime to produce the "white liquor" with which the wood is cooked. In practice a measured quantity of unrecovered black liquor is introduced into the digester together with the white liquor. This is especially true when the pulp is not to be bleached. The length of cooking is slightly longer than that required for soda pulp but a longer and stronger fibre is produced.

Sulphate pulp is often of a brown colour and difficult to bleach but being particularly adapted to the manufacture of wrapping papers finds a ready market as an unbleached pulp. Originally "kraft" was a brown paper made from a slightly undercooked soda or sulphate pulp but the term has been so much abused that its present meaning is any strong paper, irrespective of colour, made from sulphate or sulphite fibre.

Red Ash and White Liquor.—The total alkalinity of the "red ash" or of the "white liquor" can be measured by dissolving a sample in water and titrating with normal acid using methyl-orange as indicator. The estimation of sulphides, sulphites, thiosulphates and sulphates in the solution presents great difficulty.

[1] *Eighth Intern. Congr. Appl. Chem.*, Vol. 13, p. 265.

W. Field[1] suggests distilling a sample with magnesium chloride in an atmosphere of carbon dioxide and collecting the hydrogen sulphide evolved in a solution of $N/10$ iodine. The excess of iodine solution is estimated by titration with sodium thiosulphate and the percentage of sulphide then estimated.

Hydrochloric acid can now be introduced into the same solution and the sulphite will be decomposed with the evolution of sulphur dioxide which is passed into $N/10$ iodine and the titration made as in the case of sulphides. Thiosulphates are estimated in a fresh sample by first titrating with $N/10$ iodine which converts sulphides to sulphur, sulphites to sulphates and thiosulphates to tetrathionates. The tetrathionate is decomposed in an atmosphere of carbon dioxide with nascent hydrogen, generated from aluminum and hydrochloric acid, and the hydrogen sulphide evolved collected and estimated as in case of sulphides.

A second somewhat similar method by Richardson and Aykroyd[2] gives perhaps more accurate results but is longer.

Carl Moe[3] suggests a rapid method of estimating the sulphide alone. A 1 c.c. sample is titrated with a solution of silver nitrate, added slowly and with repeated shaking until the precipitation of black sulphide ceases and a turbidity in the solution just disappears. The titration should be performed over a white surface to obtain the best results. The writer suggests a solution containing 55.81 grm. of metallic silver or 87.89 grm. of silver nitrate to the litre. 1 c.c. of this solution is equivalent to 1 pound of sodium oxide (Na_2O) to the cubic foot of solution.

Sulphite pulp is produced when small chips of wood are treated at a high temperature and pressure with calcium hydrogen sulphite solution. The chips should be uniform in size, free from bark and sawdust, their length depending on the method of cooking to be employed. In general a longer chip can be employed for the manufacture of an unbleachable pulp than in the case of one to be bleached. The best results are obtained when dry wood is employed, although it is possible to "cook" green wood. .

The "acid" used is an aqueous solution of calcium and magnesium hydrogen sulphites in which is dissolved an excess of sulphur dioxide. The acid is prepared by burning either sulphur or iron pyrites and absorbing the gas in cold milk of lime. In burning either, close control over the admission of air should be exercised. Too rapid introduction of air, or leaks in the absorbing apparatus, always results in the formation of sulphur trioxide and consequent loss in efficiency. Furthermore a deposit of calcium sulphate forms in the pipe lines and clogs them. If the supply of oxygen is insufficient for complete combustion sulphur distils over and is deposited throughout the system; this must later be removed.

There are two distinct systems of acid making, the limestone tower and

[1] *Dic. Chem. Ind.* (1898), 372.
[2] *J. Soc. Chem. Ind.*, 1896, 15, 171.
[3] *Paper*, 1914, 14, No. 22, 19.

the milk of lime system. In the limestone tower system, blocks or lumps of dolomite or of limestone are charged into towers or tanks. Water enters at one end while sulphur dioxide gas is introduced and finished liquor is discharged from the other. In the milk-of-lime system, lime milk is introduced into the towers or tanks and sulphur dioxide gas bubbles through it. In some systems the gas produced by burning sulphur is materially strengthened by the introduction of gas relieved from the digester. In others the relief gas is introduced into the acid liquor storage tanks.

The digestion of the wood is effected by two well-defined methods, the Mitscherlich or "slow cook" and the "quick cook." The former is used extensively in Europe while the latter conforms more commonly to American practice. In the slow cook the wood is steamed for a period, after which the acid liquor is introduced and the actual digestion takes place; the heating being by indirect steam. At the beginning of the cook it is possible to introduce live steam to hasten the action. In making a quick cook the acid and chips are introduced together into the digester, the cover securely fastened and live or superheated steam introduced until the maximum conditions of temperature and pressure are obtained. These are assigned values, since they vary with the different grades of pulp that a mill may produce, but are constant for any single grade.

The pulp from the digester is thoroughly washed, screened and made into laps or folds for "unbleached," or passed into bleachers if it is to be made into "bleached" sulphite pulp.

Sulphite pulp possesses a long, strong fibre and is used largely in the manufacture of wrapping papers and as a binder for mechanical fibre in newspaper. The bleached sulphite is used in the manufacture of writing papers and for similar purposes.

Acid Liquor.—The percentage of free sulphur dioxide is estimated by introducing a 1 c.c. sample into a volume of not less than 100 c.c. of water. A few drops of phenolphthaleïn are added and the solution titrated with $N/10$ sodium hydroxide. The number of c.c. of alkali used multiplied by 0.32 gives the percentage of free sulphur dioxide in the liquor.

The total sulphur dioxide is estimated by titrating a 1 c.c. sample diluted with water as in the preceding case with $N/10$ iodine using starch as indicator. The number of c.c. used multiplied by 0.32 gives the percentage of total sulphur dioxide. The combined sulphur dioxide is obtained by subtracting the percentage of free from the percentage of total sulphur dioxide.

Pyrites vary in sulphur content from 35 to 50%. It is therefore necessary to value each parcel on the basis of available sulphur. A sample, finely ground, is treated with nitric acid and bromine water to oxidize the sulphur to sulphate. The volume is then rendered large to prevent occlusion of iron and a 10% solution of barium chloride introduced into the boiling

solution as precipitant. The barium sulphate is filtered off, dried and weighed and the percentage of sulphur present estimated.

Sulphur can usually be purchased with a guarantee of from 98.5 to 99% purity. It is seldom analysed, although it is customary to check the moisture content. This is done by drying a 10 grm. sample *in vacuo* over sulphuric acid to constant weight.

Lime should contain a high percentage of magnesium. This is because magnesium has a higher combining value, *i.e.*, lower atomic weight, than calcium and because the sulphites and sulphates of magnesium are more soluble than those of calcium. Impurities in lime which affect adversely the quality of paper pulp are iron and silica. A lime of good quality gives the following analysis: CaO 56%, MgO 42%, Fe_2O_3 and Al_2O_3 0.5 %, SiO_2 1.0%, CO_2 and volatile matter 0.5%. For a limestone tower system the same conditions apply and the same impurities are detrimental to the quality of the pulp.

Sulphate in acid liquor is usually estimated by adding to a 25 c.c. portion of the liquor 10 c.c. of pure hydrochloric acid, heating the solution to boiling and adding drop by drop 15 c.c. of 10 % barium chloride. The precipitation of calcium sulphite is prevented by the strong acid. The precipitate is filtered, washed, ignited and weighed. Convert by factor to per cent. $CaSO_4$. In this estimation the amount of sulphate is reported either as pounds per digester charge or as grains per gallon of liquor.

Sulphur dioxide in gas is estimated by means of an Orsat apparatus using sodium hydroxide as an absorbing agent. The use of mercury in the measuring burette may be omitted if water thoroughly saturated with sulphur dioxide is substituted.

Oxygen.—In order to regulate the draughts in the burner it is often advisable to know the percentage of oxygen in the gas since this is always a diluent and may cause loss in sulphur by oxidising some of the gas to the trioxide. This estimation is made with the Orsat apparatus, alkaline pyrogallic acid solution being used as an absorbing agent, after the sulphur dioxide is removed by the sodium hydroxide.

Iron in Pulp.—Iron is always present to varying extents in pulp. In order to keep this value at a minimum the pulp should be examined for specks of iron. The sheet is held between the observer and a bright light and suspicious places are marked with a pencil. The pulp is cut open until the particle is exposed, two drops of nitric acid are added and a drop of a 10% solution of potassium ferrocyanide or of potassium thiocyanate, when the presence of iron will be evident. The presence of shives is noted by holding the pulp sheet to the light and reporting the number in the average sheet.

Mechanical or ground-wood pulp is produced by grinding a log against the surface of a revolving stone. The logs are placed parallel to the axis of the stone, against the periphery of which they are held by hydraulic pressure. The quality of the pulp depends on the uniformity of the fibres and

its freedom from bark, shives and mechanical dirt. There are five factors[1] that influence the quality of mechanical pulp: (1) the proper dressing of the surface of the stone, regulating sharpness and grit; (2) the pressure with which the wood is held against the revolving stone; (3) the peripheral speed of the stone; (4) the temperature of grinding; (5) the quality of the wood employed.

When the stone is very sharp the fibres are literally ground to pieces, a large percentage of shives or slivers is made, and the pulp produced is of extremely short fibre. With a dull stone a pulp with long fibre and few shives is produced, although a greater expenditure of horse-power is required. The effect of increase in pressure in the cylinder and of increase of peripheral speed of the stone are identical. The production of pulp is increased and the strength factor and horse-power per ton of pulp are reduced. Change of peripheral speed is far less effective than increase in pressure.

The question of temperature is differently considered by European and American manufacturers. The cold grinding process producing a fine pulp, free from shives and quite opaque is extensively used by European manufacturers. American practice consists of hot grinding which produces long and strong fibres. In cold grinding about 500 pounds of water are used to one of pulp; in hot grinding the ratio is 50 to 1. The effect of quality of wood is obvious and requires no comment.

Mechanical pulp is used principally in newspapers where strength and permanence are not important factors. It also finds use in the manufacture of wall board and products of similar character. The pulp has a slightly yellow colour and is bleached only with great difficulty and at high comparative cost, so that bleaching is seldom attempted. The yellow colour is counteracted by the addition of dyes, either blue alone or red and blue.

A typical newspaper furnish would be: mechanical pulp 675 pounds, sulphite pulp 225 pounds, talc 45 pounds, clay 45 pounds, soluble blue 4 ounces, rhodamine ½ ounce.

Paper pulps are characterised as easy or as hard bleaching according to the amount of chloride of lime required to bring the pulp to a standard colour or quality. An easy bleaching pulp has been defined as any pulp that will bleach with 12% or less of bleaching powder.[2]

The action of the bleach liquor is on the ligneous matter left in the wood after the digestion, to oxidise and destroy it. An excess of bleach liquor attacks the cellulose itself forming oxycellulose, which is of no value as a fibrous material.

Heinz C. Lane[3] suggests the following qualitative tests based on the fact that oxidising agents such as potassium permanganate, hydrogen peroxide and potassium dichromate produce a red colour with the organic matter other than cellulose in pulp. The test consists in pouring a definite quantity, 3 or 5 c.c. of a solution containing 0.25 grm. of $K_2Cr_2O_7$ and 10 c.c. of N-hydro-

[1] U. S. Forest Service, *Bul.* No. 127.
[2] *Pulp and Paper Magazine of Canada*, 1914, 12, 203.
[3] *Pulp and Paper Magazine of Canada*, 1914, 12, 430.

chloric acid to the litre, on to uniform samples of pulp and allowing them to stand for 2 to 3 minutes when the colour will have developed. From the shade developed the amount of bleach necessary can be estimated.

Sindall and Bacon[1] suggest the use of a tintometer for measuring shades and that these be made permanent by matching them on porcelain plates so that a permanent and uniform standard may be maintained.

It is often necessary to know the exact point to which the action in the bleaching vat has progressed. To this end titrations of the bleach liquor should be made at intervals of 1 hour at the beginning of the bleaching, progressively shortening to 15-minute intervals toward the end the values should be plotted with time as ordinate and either grains of bleach liquor or c.c. of arsenious acid solution as abscissa.

The method of titration consists of withdrawing 50 c.c. samples with a pipette and introducing them into a casserole. A few drops of starch potassium iodide solution are added and the solution titrated with $N/10$ arsenious acid until the blue colour disappears.

Making of Sulphite Pulp.—The process is controlled by analysis of the digester liquor for contained sulphur dioxide. At intervals of an hour during the first part of the "cook," and at increasingly shorter intervals as the "cook" progresses, a portion of at least 600 c.c. is drawn from the sample tap on the side of the digester. From this hot sample 5 c.c. are quickly added by a pipette to a volume of cold water of not less than 250 c.c. The sulphur dioxide is estimated by titrating with starch and $N/10$ iodine. The percentages of sulphur dioxide found determine the time of emptying the digester.

More than 0.40%; the pulp is not ready for emptying.

0.38 to 0.34; the pulp *can* be emptied but is somewhat coarse.

0.32 to 0.27; the pulp is ready if not required for bleaching.

0.26 to 0.13; the pulp is ready if required for bleaching.

0.11 to 0.05; the pulp begins to become discoloured.

0.03 to 0.02; the pulp is "burnt."

The method is approximate in that there is a considerable loss of sulphur dioxide from the hot solution, but the loss is nearly constant and so the method has been found satisfactory in practice. A few workers avoid this error by drawing the sample through a water-cooled condenser. The percentages obtained by this last method of sampling are very materially higher.

Special Tests.—The following should be consulted by those interested in the subjects indicated as the space available does not permit their detailed discussion here.

A method of measuring numerically the "handle" (German "Griff") of a paper. Briggs, *Papier Fabr.*, 1914, 12 (Convention Number 25a), 27.

Testing the beating time of pulp. Stark, *Papier Fab.*, 1913, 11, 1358.

The copper figure and the true acid figure of sulphite and other cellulose as

[1] *Pulp and Paper Magazine of Canada*, 1914, 12, 203.

a measurement of the dyeing, sizing and bleaching qualities of a pulp. Richter, *Pulp and Paper Mag. Can.*, 1914, **12**, 193.

Specifications.—The following is a specification of a paper for the United States Government and is typical of American practice.

Machine-finish Printing Paper No. 1.

(12,000,000 pounds to be supplied in rolls and flat of specified standard sizes.)

Weight.—25 × 40 in., 500 sheet; 44 pound basis (ream).

Thickness.—Shall not exceed 0.0035 in.

Strength.—Shall not be less than 12 points (as given by the Mullen bursting strength tester).

Stock.—May be bleached chemical wood, free from unbleached or groundwood pulp.

Ash.—Shall not exceed 10 per cent.

Colour, Finish, Formation and Opacity.—Deliveries must conform to standard sample.

ERRATA IN VOL. I.

Page 471, Fig. 76 title should read "Mullen's" instead of "Muller's."
Page 476, line 23 should read "or" instead of "of."

ALIPHATIC ACIDS.

By ARTHUR W. THOMAS and W. A. DAVIS.

ACETIC ACID AND VINEGAR.

Acetic Acid.

Physical Properties.—According to Bousfield and Lowry[1] acetic acid is best purified by distilling from potassium permanganate, using a still head to retain the acids of higher boiling point, and then freezing to remove the water. The purified acid melts at 16.60° and has a sp. gr. 1.05148 at 18°/4° and 1.04922 at 20°/4°; its maximum conductivity when mixed with water is $\kappa_{18} = 0.0016415$. The boiling point of the pure acid is 117.88° ± 0.05° under 760 mm.[2]

According to Orton, Edwards and King[3] the degree of freedom of acetic acid from impurities is best judged by determining the fall in titre of a solution of bromine in the acetic acid diluted with an equal volume of water. For this purpose a standard solution of bromine (about $N/5$) is made in pure acetic acid; 5 c.c. of this solution are added to 45 c.c. of the acetic acid to be tested and 50 c.c. of water are introduced. The mixture is placed *in the dark* in a bath at 16° and the free bromine determined at intervals with hydriodic acid and $N/50$ thiosulphate.

Impurities.—For the *detection and estimation of acetic anhydride* when present in acetic acid in small quantities Edwards and Orton[4] make use of the fact that 2:4 dichloroaniline combines rapidly with acetic anhydride but not with acetic acid at the ordinary temperature. The resulting anilide is readily and quantitatively converted into a chloroamine and the accurate estimation of the latter is extremely easy owing to its quantitatively liberating iodine from hydriodic acid, the iodine being determined as usual by means of thiosulphate.

The process is as follows: 2 grm. of 2:4-dichloroaniline (or a quantity equivalent to about 3 times the amount of the acetic anhydride supposed to be present) is added to 100 c.c. of the acetic acid to be tested. The mixture is left overnight in a bath at 16°.

The mixture is then diluted with water so as to contain about 20% of

[1] *Trans.*, 1911, 99, 1432.
[2] Ordinary acetic acid contains traces of glyoxylic acid and hence gives the Adamkiewics reaction (Hopkins and Cole, *Proc. Roy. Soc.*, 1901, 68, 21); the acid purified in the manner described above is stable to bromine and does not give the Adamkiewics indication. Deterioration of such acid does not occur even when the acid is exposed to light or atmospheric oxidation.
[3] *Trans.*, 1911, 99, 1178.
[4] *Trans.*, 1911, 99, 1181.

acetic acid and the aniline (partly) and the anilide (wholly) extracted with chloroform. For each 100 c.c. of the diluted liquid first 10 c.c., secondly 7 c.c. and lastly 5 c.c. of chloroform are used. The unchanged aniline is now withdrawn from the chloroform by shaking for a few minutes with 10% hydrochloric acid, 160–200 c.c. being used for each gram of the aniline originally present. The chloroform is carefully drawn off in a separating funnel and the hydrochloric acid washed twice with 1 c.c. of chloroform. The chloroform solution is now mixed with an equal volume of pure acetic acid and dilute bleaching powder solution slowly added (3 to 5 times that required to convert the anilide to chloroamine). The volume of the bleaching powder solution should be equal to the volume of the glacial acetic acid, that is the acetic acid is diluted to 50%, a concentration at which the formation of chloro-amine is complete. An $N/20$ bleaching powder solution is now added in sufficient quantity to dilute the aqueous acetic acid to 20% acetic acid. For example if 30 c.c. of glacial acetic acid were added to the chloroform solution of the anilide, then 30 c.c. of bleaching powder solution would be required for the first addition and 90 c.c. of $N/20$ bleaching powder for the second. Chloroform withdraws the chloroamine completely from 20% acetic acid. Any marked development of red colour at this stage shows that the aniline has not been properly separated.

· The chloroform is drawn off into 30 to 40 c.c. of $N/20$ bleaching powder solution, with which it is shaken to free it from acetic acid. Finally the chloroform is evaporated in a current of pure dust-free air at a temperature not exceeding 25°. The residue is dissolved in chloroform (5–10 c.c.) and treated with 5% potassium iodide and a little acetic acid; the iodine liberated is titrated with $N/20$ thiosulphate.

Formic Acid in Acetic Acid.—Ost and Klein in 1908 pointed out that commercial acetic acids usually contain formic acid, different samples showing various proportions up to 0.6%, and Pikos showed that it was quite easy commercially to produce acetic acid free from this impurity. In spite of this Fincke[1] states that formic acid is still an impurity which is usually present (0.018 to 0.806%) in the acid used for pharmaceutical purposes. To *estimate the formic acid* present, Fincke operates as follows: 5 c.c. of the glacial acid, 5 grm. of sodium acetate, 40 c.c. of 1 in. 20 solution of mercuric chloride and 30 c.c. of water are heated in an Erlenmeyer flask fitted with a reflux condenser for 2 hours in a water-bath, the part of the flask containing the liquid being fully immersed. The precipitated calomel is collected in a Gooch crucible, dried and weighed; its weight multiplied by 0.0977 gives the weight of formic acid.

Aluminium Acetate.—Enz[2] states that a supply of dilute acetic acid (sp. gr. 1.041) used in the preparation of pharmaceutical products contained 0.095% of alumina in the form of aluminium acetate.

[1] *Apoth. Zeit.*, 1910, 727.
[2] *Apoth. Zeit.*, 1912, 27, 942.

According to the new British Pharmacopœa (1914) the following are the requirements for acetic acid (glacial). It crystallises when sufficiently cooled and does not entirely remelt until the temperature rises above 14.7°. 1 grm. diluted with 50 c.c. of water requires for neutralisation not less than 32.9 c.c. of $N/2$ solution of sodium hydroxide. It leaves no residue on evaporation and yields no characteristic reaction for lead, copper, arsenic, chlorides, nitrates, sulphates or sulphites. It does not immediately darken in colour when neutralised with *solution of ammonia* and warmed with *solution of silver nitrate* (absence of formates). 2 c.c. of glacial acetic acid do not completely decolourise a mixture of 3 drops of *solution of potassium permanganate* and 10 c.c. of water within half a minute (limit of empyreumatic matter.)

Vinegar.

Russell and Hodgson[1] give the following analyses of 12 samples of genuine malt vinegar and 2 samples of wood vinegar.

	Malt vinegars		Wood vinegars	
	Lowest	Highest	1	2
Sp. gr.	1013.7	1022.1	1012.1	1012.4
Acetic acid, %	3.85	6.36	2.20	2.30
Total solids, % ...:.............	1.47	3.15	0.47	0.43
Ash, %	0.18	0.60	0.04	0.03
Alkalinity of ash (as K_2O %)......	0.016	0.040	0.014	0.022
P_2O_5, %	0.047	0.092	Nil.	Nil.

In estimating total solids it was found that the residues retained from 0.15 to 0.22% of acetic acid and that a neutral residue was obtained only when the latter was moistened with water and evaporated 3 times in succession. It is suggested that 3.5% of acetic acid and 0.05% of phosphoric acid should be taken as the standards for malt vinegar.

Volatile Acids in Wines and Vinegars.—Gore[2] describes the apparatus shown in Fig. 4 for the estimation of volatile acids in wines and vinegars. The apparatus is a modification of that due to Hortvet-Sellier, in which a copper flask is substituted for the outer glass flask and a constant-feed device for the flask has been added. A small ridge is also blown in the inner flask to form a shoulder for the rubber gasket and the dropping funnel has been eliminated as unnecessary. The constant water feed is operated by running the supply water through tube *a*, which passes through tube *b*. The overflow passes through *b* and rises through *c* to the small basin *d* which is connected to the drain. Distilled water should be used which has been largely freed from carbon dioxide by passing a rapid stream of air through it for 20–30 minutes. The rate of flow of the water is regulated by comparing the rate of

[1] *Analyst*, 1910, 35, 346.
[2] *U. S. Bureau of Chem.*, 1909, Circular No. 44.

drip in the small sight-tube e with that from the condenser. Experiments with the apparatus showed that when tap-water was suppplied to the generator there was an error, due to the presence of carbon dioxide in the distillate equal to about 0.3 c.c. of $N/10$ alkali in 100 c.c., when phenolphthalein was used as indicator. When ordinary distilled water was used the error was 0.15 c.c., and when the carbon dioxide was removed by aeration, as de-
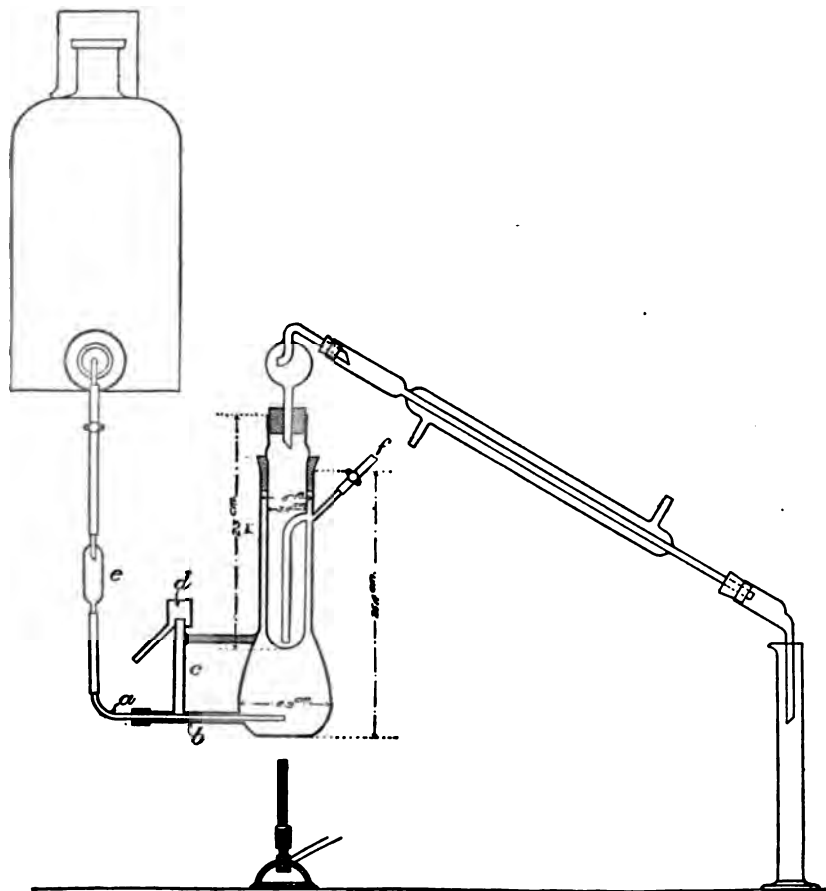
FIG. 4.—Modified Hortvet-Sellier apparatus.

scribed, the error was only 0.05 c.c. This error is constant and may be subtracted from the results. The operation is carried out as follows: 10 c.c. of the sample is placed in the inner flask which should be removed from the outer flask and be quite cool. If the sample is noticeably charged with carbon dioxide, this should be removed by passing a current of air through it, by attaching to the flask a stopper fitted with a glass tube connected with suction. The air passes in through the side tube of the flask and washes out the

carbon dioxide without removing appreciable quantities of volatile acids. The flask is then connected with the distilling bulb, and placed in the outer flask, tube f of the latter being open, all connections are made tight and the tube f is then closed. In the case of wines about 100 c.c. of the distillate are collected, in the case of vinegars 200 to 300 c.c. The distillate is titrated with standard alkali, free from carbonate, using phenolphthalein as indicator, and 0.05 c.c. is subtracted from the number of c.c. of $N/10$ alkali used for each 100 c.c. of distillate. About 15 minutes are required for the estimation of volatile acid in wine, and from 30 to 45 minutes in the case of vinegar. The volume of liquid in the inner flask increases but slightly during the determination.

Mineral Acids.—According to Repiton[1] a portion of the sample is titrated with a solution of calcium saccharate, using phenolphthalein as indicator, to ascertain the total acidity. Another portion is neutralised with sodium hydroxide, free from carbonate, then acidified with phosphoric acid and subjected to distillation until the distillate is no longer acid. The distillate is then titrated with calcium saccharate; if the result obtained is identical with the total acidity, free mineral acids are probably not present. If there be a difference, sulphuric acid and hydrochloric acid should be determined in the ordinary way. Nitric acid is not likely to be present. Phosphoric acid is estimated by treating a portion of the vinegar with calcium carbonate, evaporating to dryness, incinerating and precipitating the nitric acid solution of the ash in the usual manner.

Wine Vinegar.—Fleury[2] states that as inositol is not decomposed during the acetous fermentation, its presence can be used as a test for the genuineness of wine vinegars, which always give a decided reaction of inositol. 100 c.c. of the vinegar are evaporated nearly to dryness, the residue is taken up with 50 c.c. of water, neutralised with sodium hydroxide and triturated with 3 grm. of barium hydroxide. The liquid is separated and the precipitate washed with 20–30 c.c. of baryta water. The liquid and washings are freed from barium by means of carbon dioxide or sulphuric acid and the defecation is completed by adding 10 c.c. of normal lead acetate solution (1:3). The clear liquid is made up to 100 c.c. and 10 c.c. of official basic lead acetate and a solution of 2 grm. of neutral cadmium nitrate added. The precipitate, which contains the inositol, is decomposed with hydrogen sulphide and the filtrate evaporated to a syrup. The residue is taken up with 20 c.c. of absolute alcohol and 5 c.c. of dry ether added. After 1 or 2 days' standing, the solution is tested for inositol by the usual methods.

Cider Vinegar.—From a study of fine cider vinegars F. E. Mott[3] concludes that there is a relation between the contents of lævulose and of dextrose therein. If the amounts of lævulose and dextrose (percentage of total solids) be plotted on squared paper as abscissæ and ordinates respectively, then a line

[1] *Monit. Scient.*, 1909, **23**, 172.
[2] *J. Pharm. Chim.*, 1910, **2**, 264.
[3] *J. Ind. Eng. Chem.*, 1911, **3**, 747.

drawn from the zero point through the point corresponding to 15% dextrose and 25% of lævulose will represent the maximum values for dextrose in relation to the corresponding values for lævulose in fine cider vinegar, and a higher value for dextrose indicates adulteration with a foreign dextro-rotatory substance.

In estimating the lævulose and dextrose the following method is recommended: The total solids from 10 c.c. of the vinegar are dissolved in 25 c.c. of water, the solution made up to 100 c.c. and the reducing sugars estimated by Munson and Walker's method (see page 28). 50 c.c. of the vinegar are then treated with 5 c.c. of basic lead acetate solution and two teaspoonfuls of animal charcoal, filtered and the filtrate polarised in a 200 mm. tube at 20°. From the polarisation (p) expressed in Ventzke degrees and the percentage of reducing sugars (R) in the total solids, expressed as dextrose, the percentage of lævulose (L) and of dextrose (D) are calculated by means of the formulæ.

$$P = \frac{P \times 26.048}{\text{total solids}}; \quad L = \frac{0.793 \; R - P}{2.08}; \quad D = R - 0.915L$$

Mott claims that pure cider vinegar contains about 0.6 as much dextrose as lævulose, consequently if a suspected sample shows a dextrorotation of polarised light at 87° it may be adulterated with dextrorotatory substances, usually glucose. On the other hand, a lævorotation at 87° is not conclusive evidence of purity. Mott estimates the reducing sugars by the polarimeter and by Munson and Walker's method after evaporation to remove aldehydes.

Adulteration of Cider Vinegar with Distilled Vinegar.—Pure cider vine-gars contain volatile constituents which have a reducing action on Fehling's solution. These bodies are not present in distilled vinegar or pyroligneous acid. A method based on this fact is given by S. L. Crawford[1] as follows: 50 c.c. of the sample are diluted to 250 c.c., placed in a distilling flask, and 200 c.c. are distilled into a 250 c.c. flask. The distillate is then neutralised, made up to 250 c.c. and 50 c.c. are taken for estimation of the reducing substances by Munson and Walker's method. Calculated to the basis of a vinegar containing 4% acid, the distillate should contain from 0.11% to 0.15% of reducing substances calculated as invert sugar. Pure cider vine-gars usually contain 0.007 grm. of formic acid per 100 c.c. Pyroligneous acid would raise this figure while distilled vinegar would lower it.

Lead Number.—The qualitative detection of adulteration of cider vinegar by measurement of the precipitate formed by lead acetate is regarded by many to be one of the most useful of tests. For this purpose a rapid process is given by Hortvet[2] as follows:

To 25 c.c. of the vinegar are added 2.5 c.c. of U.S.P. lead subacetate solu-tion. The mixture is shaken and whirled in a graduated Hortvet tube in a centrifugal machine. The volume of the precipitate in the bottom of the tube

[1] *J. Ind. Eng. Chem.*, 1913, 5, 845.
[2] *J. Amer. Chem. Soc.*, 1904, 26, 1532.

is then read off and this volume in c.c. is termed the *Hortvet lead number.*
The lead numbers of pure cider vinegars have been found to range from 0.5
to 1.4 while adulterated cider vinegars give numbers from almost zero to 0.5,
rarely more than 0.5.

The Hortvet tube is 15.3 cm. in length, and consists of a wide cylindrical
portion 3 cm. in diameter, narrowed at the top to a neck which is 2 cm. in
diameter, and at the bottom to a stem graduated in tenths to 5 c.c. Hort-
vet's centrifugal machine had a radius of 18.5 cm. and was run at a speed of
1600 r.p.m.

Winton's method[1] of estimating the lead number of vinegars has the ad-
vantage of not requiring the use of a centrifugal machine. This method gives
most satisfactory results but requires much more time than the Hortvet
process, owing to the fact that the lead is estimated by a gravimetric method.
Cider vinegars give Winton numbers of 0.075 to 0.290; malt vinegars much
higher values, whilst distilled vinegars give much lower figures.

Caramel in Vinegar.—L. Ronnet[2] gives the following method to detect
caramel in vinegar. 50 c.c. of the vinegar are mixed with an excess of cal-
cium carbonate and evaporated to dryness. The mass is extracted with 20
c.c. of ether, the ethereal solution is filtered and the filtrate received in a test-
tube containing 10 c.c. of resorcinol reagent (1 grm. of resorcinol dissolved in
100 c.c. of hydrochloric acid of sp. gr. 1.125). If caramel is present in the
vinegar a red zone is formed at the junction of the two liquids. Methods
which depend on heating the vinegar with albumin and extracting the evapo-
rated solution with ether are untrustworthy as traces of furfural compounds
(on which the colouration with the resorcinol reagent depends) may be formed
during the evaporation, should the vinegar contain sugars.

Miss A. Anderson[3] obtained positive tests for furfural by the aniline ace-
tate method in 19 out of 28 samples of *pure* cider vinegar. She also obtained
indications of caramel by using Fiehe's resorcinol reagent when no caramel
had been added. Hence caution must be exercised in the interpretation of
results. These observations emphasise the necessity of modifying the test in
some such manner as suggested by Ronnet, owing to the production of furfural
derivatives from sugars (compare page 64).

<div style="text-align:center">

Pyroligneous Acid.

</div>

Samples of crude pyroligneous acid containing respectively 8.4 and 8.7%
of acid calculated as acetic acid, were found by G. Frerichs[4] to give distillates
containing only 3.9 and 4.2% of acetic acid. From a comparison with older
samples it is concluded that crude pyroligneous acid as now sold contains
acids other than acetic acid and is of inferior quality to that obtained form-

[1] *J. Amer. Chem. Soc.*, 1906, 28, 1204.
[2] *Ann. Falsif.*, 1912, 5, 517.
[3] *J. Ind. Eng. Chem.*, 1914, 6, 214.
[4] *Apoth. Zeit.*, 1913, 28, 525.

erly or may consist of a waste product from which much of the acetic acid has already been distilled.

Formic Acid.

In testing for formic acid in distillates it must be borne in mind that the distillation of sugars with acids produces formic acid.

It is also dangerous to apply either the silver nitrate or mercuric chloride tests for formic acid to distillates, inasmuch as other volatile substances will give a positive indication with these reagents. Consequently it is advisable to convert the formic acid into formaldehyde by means of magnesium and sulphuric acid and then apply a convenient test for formaldehyde.

Oxalic Acid.

J. M. Albahary[1] proposes a new method of estimating *oxalic acid* in cocoa and chocolate which gives more satisfactory results than other existing processes and is also applicable in the analysis of plant juices. It is based on the fact that magnesium salts precipitate phosphates and purine bases in alkaline solution whilst oxalates remain dissolved as magnesium alkali oxalates.

The procedure is as follows:

50 grm. of the sample are dried over sulphuric acid to constant weight and then heated on a water-bath for 1 hour with 50 c.c. of 10% sodium carbonate solution, the volume being kept constant during the heating by adding water from time to time. The mixture is then treated with 50 c.c. of a solution containing 10% of magnesium chloride and 20% of ammonium chloride, a quantity of animal charcoal being added to retain mucilaginous substances. After concentrating by heating for 1 hour, with occasional shaking, the mixture is filtered hot by means of suction, and the residue washed with boiling water. The filtrate is concentrated, made strongly alkaline with ammonia and after standing 12 hours is filtered. The filtrate is treated with a slight excess of calcium chloride and made faintly acid with acetic acid. After standing 12 hours the calcium oxalate is filtered off and the oxalic acid determined in the usual way. This gives total oxalates. If the preliminary treatment with sodium carbonate be omitted, the final result will give soluble oxalates and the difference between the two results gives the amount of calcium oxalate present.

Succinic Acid.

After a critical study of all the existing methods for the estimation of *succinic acid in wine*, C. von der Heide and H. Steiner[2] recommend that the following method be used when accurate results are desired:

[1] J. Soc. Chem. Ind., 1909, 28, 738.
[2] Zeit. Unters. Nahr. Genussm., 1909, 17, 291. Zeit. Anal. Chem., 1912, 51, 628.

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50 c.c. of wine are evaporated in a 200 c.c. casserole to remove all alcohol. After adding 1 c.c. of 10% barium chloride solution and phenolphthalein the solution is neutralised with powdered barium hydroxide, the excess of barium is removed by means of carbon dioxide and then 85 c.c. of 95% alcohol are added to the mixture whilst constantly stirring. After 2 hours the precipitate of barium succinate, tartrate, malate and other barium salts is collected on a filter, washed with a small quantity of 80% alcohol and then washed back into the casserole with hot water. The solution is concentrated to remove all alcohol, 5% potassium permanganate solution is then added in 3 c.c. portions until the red colour persists for 5 minutes. An additional 5 c.c. of permanganate is added and the mixture heated on a water-bath for 15 minutes. The excess of permanganate is reduced with sulphurous acid and after acidifying with sulphuric acid more sulphurous acid is added until the manganese dioxide is dissolved. The mixture is then evaporated to about 30 c.c. and after acidifying with sulphuric acid until about 10% of free acid is present is extracted with ether for 12 hours in a percolating apparatus. The ethereal solution is evaporated with water and the residual aqueous solution is made alkaline with barium hydroxide. The mixture is then heated on a water-bath for 10 minutes, excess of barium is precipitated as carbonate, and the solution cooled and filtered. 20 c.c. of $N/10$ silver nitrate are added to the solution in a 100 c.c. flask and diluted to the mark. After standing 2 hours the excess of silver in a 50 c.c. portion is titrated by the Volhard method.

Malic Acid.

Estimation of Malic Acid.—A method of estimating malic acid in fruit juices is described by P. B. Dunbar and R. F. Bacon.[1] This method has been supplemented by D. S. Pratt[2] with a preliminary treatment which removes several mechanical difficulties in the original process. The modified method is carried out as follows:

A weighed amount of juice, generally 100 grm., is placed in a 500 c.c. beaker. With vigorous stirring about 2 or 3 times the volume of 95% alcohol is added. This throws out the pectin bodies, usually in such a form that after standing a few minutes they may be gathered into a coherent mass. The liquid is decanted through a filter and the precipitate washed twice with 95% alcohol. The combined filtrates are then evaporated in a current of air on the water-bath to about 75 c.c. After cooling, the solution is transferred to a 100 c.c. measuring flask, using 10 to 15 c.c. of 95% alcohol, sufficient sodium hydroxide is added to neutralise the solution and then the volume is adjusted to 100 c.c. The temperature when the volume is made up to the mark should be close to that at which the polarimeter readings are to be taken.

[1] *U. S. Dept. Agr., Bur. Chem., Circular, 76. J. Ind. Eng. Chem., 1911, 3, 826.*
[2] *U. S. Dept. Agr., Bur. Chem., Circular 87.*

25 c.c. of this solution are treated with about 2.5 grm. of powdered uranyl acetate. In case all the uranyl acetate should dissolve in 2 hours, more should be added. This mixture is shaken vigorously at frequent intervals during the 2-hour period and then filtered through a folded filter until clear and is polarised in a 200 mm. tube against white light in a saccharimeter. If the alcohol has caused some fruit colour to pass into solution the addition of a tiny drop of bromine and vigorous shaking and filtering, if necessary, before reading will give satisfactory results. This solution and reading are designated A.

The remainder of the original filtrate is treated with powdered normal lead acetate until the precipitation is just complete. An excess of lead acetate should be avoided so as to obviate dissolution of lead malate. This mixture is cooled in an ice box and filtered until clear. The clear filtrate is then warmed to room temperature and a small crystal of lead acetate added; if no precipitate forms the excess of lead is removed by addition of anhydrous sodium sulphate. This solution is filtered until clear and polarised. This reading is designated as B. Solutions which are sufficiently clear and contain less than 10% of sugar may be polarised directly without treatment with lead acetate.

If reading B is negative, a portion of solution B is treated with uranyl acetate as described for solution A and polarised. This reading is designated as C. If B is positive, reading C need not be made.

If the solutions are too dark to be read in a 200 mm. tube, a shorter tube may be used but the observed readings should be calculated to those obtainable with a 200 mm. tube. If reading C is numerically less than reading B, the latter should be discarded, otherwise reading B should be used in subsequent calculations.

The algebraic difference between this reading and reading A multiplied by the factor 0.036 gives the percentage of malic acid.

To estimate malic acid in cane and maple products the method of P. A. Yoder[1] should be used.

A colour test to distinguish between *succinic* and *malic acids* is given by W. O. deConinck[2] as follows:

Heat a concentrated aqueous solution of the acid with calcium salicylate. Succinic acid gives a permanent rose colour which does not fade on standing in sunlight for several days. Malic acid gives a similar colour, but on 15 to 20 minutes boiling it darkens and then disappears, leaving a yellowish liquid.

Tartaric Acid.

Analysis of Tartaric Acid Raw Material.—Although the analysis of raw material used in the manufacture of tartaric acid and its salts (cream of

[1] *J. Ind. Eng. Chem.*, 1911, 3, 563.
[2] *Bull. Soc. Chem.*, 1914, 15, 93.

tartar, Rochelle salt, tartar emetic) is now generally made by the "Goldenberg 1907 Method" (Vol. I, page 545) during the past 2 or 3 years the so-called "London Method" has been adopted for purposes of sale of tartars (argols, vinaccia tartar) and tartrate of lime in the London market; lees are still purchased in the London market on analyses made by the Goldenberg method.

London Method for Argols and Similar Tartars (Alambic Tartars).— 1.5 grm. of the finely divided sample are washed into a 350 c.c. beaker (tall form) with 10 c.c. of a solution of potassium carbonate (40 grm. K_2CO_3 in 100 c.c. of water); about 2 grm. of purified animal charcoal and water to make about 25 c.c. in all are added. The mixture is boiled gently for 20 minutes. The solution is then filtered by means of a vacuum apparatus, into a 550 c.c. beaker (tall form), the residue being washed with hot water until a total volume of 150 c.c. is obtained. 5 c.c. of hydrochloric acid (sp. gr. 1.1) are added and the solution concentrated to about 15 c.c. on a hot plate, avoiding actual ebullition. 4 c.c. of glacial acetic acid are then added and the mixture well stirred for 5 minutes. When the mixture is cold, 100 c.c. of 92 to 95% alcohol are added and the mixture is again stirred for 5 minutes. After the precipitated potassium hydrogen tartrate has stood for $\frac{1}{2}$ hour, it is filtered off on a filter-pump plate and washed with alcohol, exactly as in the Goldenberg method, until the washings are free from acidity (see Vol. I, page 546). The precipitate is dissolved in 200 c.c. of hot water and the solution is titrated, whilst boiling, with $N/5$ sodium hydroxide.

Phenolphthaleïn is used as indicator, and as the solutions are frequently highly coloured, it is used as an outside indicator on a clean white glazed tile. The sodium hydroxide used should be standardised with pure dry recrystalised potassium hydrogen tartrate under similar conditions.

For tartrate of lime the same method is employed but the animal charcoal can be omitted.

London Method for Vinaccia and High-grade Tartars.

1. Tartaric Acid Present as Bitartrate.—5 grm. of the sample are washed into a 400 c.c. beaker with 250 c.c. of hot water and 15 c.c. of N-sodium hydroxide. After boiling for 10 minutes, the titration is completed with N-sodium hydroxide using phenolphthaleïn as indicator.

2. Tartaric Acid Present as "Tartrate of Lime" (Calcium Tartrate).—2 grm. of the sample are ignited in a covered platinum dish or crucible at a low temperature. When charred, the residue is washed into a beaker with 5 c.c of hydrogen peroxide (10 volume), 30 c.c. of normal hydrochloric acid are added and the solution well boiled to destroy the excess of peroxide. Phenolphthaleïn is then added and the solution titrated back with N-sodium hydroxide. In this way the number of c.c. of N-HCl required to neutralise the ash of 1 grm. is obtained. From this is deducted the number of c.c. of N-sodium hydroxide required to neutralise the bitartrate present in 1 grm., as found by method 1, and the percentage of tartaric acid in the form of calcium

tartrate is calculated from the difference (each c.c. = 0.075 grm. of tartaric acid in the form of calcium tartrate).

Control of Working Conditions in Tartaric Acid Factories.—For the methods of analysis used in the control of factory operations during the manufacture of tartaric acid reference should be made to Lunge-Keane's "*Technical Methods of Chemical Analysis*," Vol. 3.

Commercial Tartaric Acid.

The following summary of the tests for *ash*, *arsenic* and *lead* is taken from the article on tartaric acid in Lunge-Keane's "*Technical Methods of Chemical Analysis*," Vol. 3.

Ash.—The limit for ash in tartaric acid crystal or powder used for pharmaceutical purposes in Great Britain in past years has been 0.05%. The Committee of Reference to the Pharmacopœia Committee (1908) (see British Pharmacopœia, 1914) has, however, recommended that this limit should be raised to 0.1%.

Arsenic is tested for by the following method recommended by the Royal Commission on arsenical poisoning (1903). From 1–5 grm. of tartaric acid are used, with arsenic-free zinc and hydrochloric acid. The limit of arsenic adopted is 1/100 grain per pound (1.4 parts As_2O_3 per million, or 0.00014%.) There is no difficulty in obtaining tartaric acid in which arsenic falls considerably below this figure.[1]

Lead.—The limit of lead generally adopted in commerce in Great Britain is 20 parts per million (0.002%), as recommended by McFadden.[2] Many methods have been suggested for making the test, of which the following are probably the most reliable:

Method I.—Based on C. A. Hill's method,[3] which is, like the following one, a modification of Warington's original colourimetric method.[4] Hill's process has been adopted by the 1914 British Pharmacopœia, and is described therein in detail.

A standard lead solution is prepared containing 5 parts of lead per million, by dissolving pure metallic lead in a minimum quantity of nitric acid (equal parts of concentrated acid and water) and suitably diluting; it is best to prepare a standard stock solution of lead, containing say 1 grm. of lead per litre, and then, when required, to dilute this for use to the above concentration. The standard solution may also be prepared from pure lead nitrate or from crystalline lead acetate.

For the test, 7 grm. of the sample are weighed out, and a separate portion of 2 grm. for the colourimetric comparison. Each portion is dissolved in about 10–15 c.c. of water, and to the 2 grm. portion are added as many c.c. of the standard lead solution as there are suspected to be parts per million of

[1] Cf. A. W. McFadden, Local Government Board Report, Inspector of Foods, No. 2, 1907.
[2] Cf. Tatlock and Thomson, *Analyst*, 1908, 33, 173; T. F. Harvey and J. M. Wilkie, *Chem. and Drug.*, 1909, 75, 92.
[3] *Chem. and Drug.*, 1905, p. 388.
[4] *J. Soc. Chem. Ind.*, 1893, 12, 97.

lead in the tartaric acid tested.   Thus to compare with 15 parts of lead per million, 15 c.c. of the standard lead solution are used.   To each solution are then added 1–2 c.c. of 10% potassium cyanide solution and 13 c.c. of ammonia of sp. gr. 0.880, and the solutions are boiled for half a minute or longer so as to get both colourless if possible.   They are next poured into two 50 c.c. Nessler cylinders of clear white glass and diluted to an equal volume (50 c.c.), any difference of colour being corrected if necessary by the addition of a drop or two of a very dilute solution of caramel.   To each solution are then added 1–2 drops of a freshly prepared colourless ammonium sulphide solution (obtained by saturating ammonia (sp. gr. 0.880), diluted with an equal volume of water, with hydrogen sulphide gas, carefully washed by passing through water).   The colour of the two solutions is compared by examining them, placed on a sheet of white paper, in a good light.

Generally it is sufficient to make sure that the quantity of lead present is less than 20 parts per million, but if it is necessary to ascertain the exact quantity, comparisons are made with suitable proportions of lead (5, 10, 15, etc., c.c. of the dilute standard lead solution).

Method II.—J. M. Wilkie's Method.[1]—7 grm. of the sample are taken for the test, and 2 grm. for the colourimetric comparison, to which the standard lead solution is added.   Each is dissolved in about 35 c.c. of hot water, allowed to cool, a few drops of $N/10$ sodium thiosulphate solution added, and heated to incipient boiling, when the flame is removed.   Any ferric iron present is rapidly reduced on cooling.   When the solution is water-white, potassium cyanide (1–2 c.c. of 10% solution) is added, and then ammonia until the solution just smells of it (excess should be avoided).   After diluting in Nessler cylinders, 2 drops of colourless ammonium sulphide solution are added and the colourations compared as described above.

In Germany, the following test, due to W. Klapproth, is used.   20 grm. of the sample are ignited with 0.04 grm. of calcium carbonate in a porcelain crucible.   The small residue (which contains all the lead) is dissolved in a few drops of nitric acid, 2 or 3 drops of sulphuric acid added, and the mixture heated to expel the nitric acid.   The residue, consisting of lead and calcium sulphates, is dissolved in ammonium acetate solution and the solution filtered from insoluble matter (ferric oxide).

To the clear solution hydrogen sulphide water is added and the resulting brown colouration compared with that of a solution of ammonium acetate in water, containing a known quantity of lead to which hydrogen sulphide water has been added under similar conditions.   To make certain that the brown colouration is due to lead and not to copper, some potassium cyanide solution is added, which destroys the brown colour due to copper, but has no effect on that due to lead.

Other Tests.—Quantities of 3 grm. of the acid are dissolved in water and submitted to the following tests.

[1] *J. Soc. Chem. Ind.*, 1908, **28**, 636; Harvey and Wilkie, *Chem. and Drug.*, July 17, 1909.

The solution of the pure acid should give no turbidity with barium chloride; nor, after the addition of nitric acid, with silver nitrate. The solution rendered alkaline with ammonia should give no precipitate with ammonium oxalate. The acid should require for titration the calculated quantity of normal alkali, which has been standardised by pure potassium hydrogen tartrate, using phenolphthaleïn as indicator, under exactly the same conditions of concentration.

British Pharmacopœia, 1914.—The requirements of the new Pharmacopœia are as follows for tartaric acid: 1 grm. dissolved in water requires for neutralisation not less than 13.2 c.c. of $N/1$ solution of sodium hydroxide. Yields no characteristic reaction for copper, iron, or oxalates and not more than the slightest reaction for calcium. *Lead limit* 20 parts per million; *arsenic limit* 1.4 parts per million. 1 grm. dissolved in 50 c.c. of water on addition of 0.5 c.c. of *solution of barium chloride* does not yield a greater opalescence than 1 c.c. of $N/100$ solution of sulphuric acid when precipitated under the same conditions (limit of sulphates); ash not more than 0.1%.

Cream of Tartar.—This occurs in commerce in different grades of purity, containing varying proportions of calcium tartrate or calcium sulphate. The usual qualities are 95, 98, and 99–100%.

The total tartaric acid is best estimated by the Goldenberg method.

The *acidity*, on which the percentage of cream of tartar is generally gauged, is ascertained by titrating 5 grm. of the sample with $N/1$ potassium hydroxide, which has been standardised by titration with 5 grm. of pure recrystallised 100% cream of tartar, dried at 100°, under exactly the same conditions of concentration, using phenolphthaleïn as indicator. The titration can also be made with 2 grm. of the cream of tartar and $N/5$ alkali standardised under the same conditions as in the actual test. The observance of exactly similar concentrations for standardisation and the actual test is necessary if exact results are to be obtained, owing to the hydrolysis of the neutral tartrate by water, which causes more alkali to be required (0.1–0.3 c.c.) in dilute solution than in concentrated solution.

To estimate tartaric acid rapidly in cream of tartar, baking-powders, etc., F. W. Richardson and J. C. Gregory.[1] and R. O. Brooks[2] have advocated a polarimetric method.

The requirements as regards *arsenic* and *lead* are the same as for tartaric acid.

Other Methods of Estimating Tartaric Acid.—It is generally recognised that the Goldenberg method, using litmus as indicator, gives slightly low results for tartaric acid when, as is usual in the case of raw material such as lees and crude tartars, phosphates of aluminium and iron are present or certain other substances such as malic acid or gummy impurities accompany

[1] *J. Soc. Chem. Ind.*, 1903, 22, 405.
[2] *J. Amer. Chem. Soc.*, 1904, 26, 813.

the tartaric acid.[1] As, however, the presence of such impurities in tartaric acid raw material causes considerable loss of the acid during its manufacture, the Goldenberg method represents, in the case of the lower grade material probably the fairest means of arriving at the value of such consignments. It must be remembered, however, that the Goldenberg process is a conventional one, and may, when absolute results are required, give rise, in presence of the impurities enumerated above to some considerable degree of error, ranging from 0.5 to 5% of the tartaric acid present, according to the proportion and nature of the impurities present.

Various other processes of estimating tartaric acid have therefore been suggested, none of which has yet been adopted for commercial purposes. A brief description of some of the most promising of these methods is here appended, as in certain special cases such methods might find a useful application.

Chapman and Whitteridge's Method.[2]—In this method, the tartaric acid is precipitated from its solution as bismuth tartrate, the latter being practically insoluble in dilute acetic acid; the barium tartrate is titrated, subsequently, in sulphuric acid solution, with potassium permanganate. The process is carried out as follows: A known weight of the substance to be analysed, containing about 0.1 grm. of tartaric acid, is dissolved in 40 c.c. of water; the solution is neutralised with either sodium hydroxide or acetic acid, as the case may require, and is then heated nearly to boiling. 15 c.c. of bismuth reagent (prepared by dissolving 30 grm. of crystallised bismuth nitrate in 20 c.c. of glacial acetic acid, diluting the solution to a volume of 300 c.c. with water, and filtering, if necessary) are added, the mixture is stirred vigorously for a few minutes, and the precipitate is collected on a filter and washed well with boiling water. The precipitate is then dissolved off the paper with about 20 c.c. of hot 10% (by volume) sulphuric acid, a further 30 c.c. of the same acid being used to wash the paper. A 1% potassium permanganate solution is then run into the hot acid filtrate until a slight excess, not exceeding 0.5 c.c., is present, and this is titrated back with oxalic acid solution containing 19.9 grm. of the pure crystallised salt per litre. As the result of many determinations, it is found that 0.1 grm. of tartaric acid corresponds with 14 c.c. of the permanganate solution. For complete precipitation, it is well to add one and a half times the quantity of bismuth reagent theoretically necessary. The method gives accurate results in the case of Rochelle salt, crude tartars, Seidlitz powders, and baking powders. Alum and succinic acid, if present, have no influence on the accuracy of the results, but the method cannot be applied to the determination of tartaric acid in the presence of citric, oxalic, and malic acids.

[1] Compare Hecsko, *Zeit. Anal. Chem.*, 1911, 50, 12. The objections which have been raised to the Goldenberg method since its adoption are based on facts which were well realised by the manufacturers and committees responsible for its acceptance as a practical test of the value of raw material on the grounds given above. The proposal by Ordonneau (*Bull. Soc. Chim.*, 1909 [iv], 7, 1034), that phenolphthalein should be used as indicator would lead to higher prices being paid for low-grade material, the tartaric acid of which cannot be completely extracted owing to the presence of alumina, phosphoric acid, etc.
[2] *Analyst*, 1907, 32, 163.

Beys' Method.[1]—Between 1 and 2 grm. of the material to be analysed is heated for half an hour on the water-bath with an equal weight of sulphuric acid diluted with 20 times its volume of water. When the liquid is cool, 5 times its volume of alcohol and 10 times its volume of ether are added, and the liquid filtered. The residue is washed with a mixture of 1 part of alcohol with 2 parts of ether. Some phenolphthaleïn is added to the filtrate, which is neutralised with potassium hydroxide dissolved in 90% alcohol. The amount of the latter is noted, and 5–10 c.c. more are added. The whole is heated till the ether begins to boil off, when it is allowed to cool. The clear liquid is poured off and excess of glacial acetic acid added. The crystals left behind are heated with 12–15 c.c. of water and 5 c.c. of glacial acetic acid are added, causing the precipitation of the greater portion of the potassium bitartrate. 30 c.c. of 96% alcohol are added, which is about the quantity required to make the alcoholic strength of the mixture 65%. After shaking for a few minutes and standing for an hour the precipitate is filtered off, washed first with 15 c.c. of 65% alcohol, then with 96% alcohol. The other precipitate produced by the addition of acetic acid (see above) is washed with 96% alcohol. Both precipitates are dissolved in the same quantity of boiling water. The liquid is then titrated as in the Goldenberg method, but using phenolphthaleïn as indicator.

Kling's Method.[2]—The errors which arise in the usual method of estimating tartaric acid by precipitation of its acid potassium salt are said to be avoided by precipitating the acid as calcium racemate, $Ca(C_4H_4O_6)_2, 8H_2O$, and titrating a solution of this with potassium permanganate. The details of the method are as follows: 25 c.c. of a solution of d-tartaric acid (containing 3–4 grm. per litre) are added to 100 c.c. of water, 25 c.c. of a solution of l-Seignette salt (16 grm. per litre: free from d-salt) and 20 c.c. of a solution of pure calcium acetate (30 grm. per litre). The precipitate is collected, washed and dissolved in 20 c.c. of hydrochloric acid (40 grm. per litre). The solution is diluted to 150 c.c. and added to 40 c.c. of a solution of sodium actetate (10%) and of calcium acetate (1%) and boiled. After cooling the racemate is collected, washed with water, redissolved in 10% boiling sulphuric acid and titrated at the boiling point with a solution of permanganate (containing about 16 grm. per litre) of which the titre has been determined by means of pure bitartrate. The method gives good results and can be used for the estimation of tartaric acid in wines.

In a later paper Kling and Florentin[3] have modified this process so as to determine with a considerable degree of accuracy, tartaric acid contained in metallic salts, tartars, and lees.

The solutions required are: (a) A solution of di-ammonium citrate, containing 50 grm. of the salt per litre; (b) a solution containing 20 grm. of pure l-ammonium tartrate (free from the d-compound (see below, page 107),

[1] *Bull. Soc. Chim.*, 1910, **7**, 697.
[2] *Compt. rend.*, 1910, **150**, 616.
[3] *Eighth Int. Congress App. Chem.*, 1912, Sect. I, 237.

and 5 or 6 c.c. of formaldehyde (as preservative) per litre; (c) a solution prepared by dissolving 16 grm. of chemically pure calcium carbonate in 120 c.c. of glacial acetic acid and diluting to 1 litre; (d) dilute hydrochloric acid containing 40 grm. of the 22° Bé. acid per litre; (e) a solution prepared by dissolving 5 grm. of calcium carbonate in 20 grm. of acetic acid, adding 100 grm. of sodium acetate and diluting to 1 litre; (f) a solution of potassium permanganate containing 16 grm. per litre. The permanganate is standardised against a solution of tartaric acid of known titre, determined with $N/10$ potassium hydroxide. In the determination of tartaric acid, in the presence of interfering metals or not, the solution is diluted to a volume of 150 c.c., 10–15 c.c. of solution a are added, and then successively 25 c.c. of solution b and 20 c.c. of solution c. The mixture is agitated and allowed to stand for several hours (12 hours if appreciable quantities of interfering metals are present). After being filtered off and washed with cold water, the precipitated racemate is dissolved in 20 c.c. of the dilute hydrochloric acid (solution d), the solution is diluted to 150 c.c., 40–50 c.c. of solution e are added, the mixture is heated to about 80° C. and then allowed to cool for several hours. The resulting precipitate is filtered off, washed, redissolved in hot, dilute sulphuric acid (10% by volume) and titrated at the boiling point with the permanganate solution.

This process was also applied by Kling and Gelin[1] to solutions prepared by heating alcoholic solutions of tartaric acid in sealed tubes and containing known amounts of tartaric acid, the latter being present in the free state and in the form of acid and neutral ethyl tartrates. The amount of tartaric acid corresponding to the total acidity being found by titration with $N/10$ potassium hydroxide, the free acid present was determined by the racemate method in a second portion of the liquid. A third portion was hydrolysed with alkali in very dilute solution, the racemate process being then applied to estimate the total (free and combined) acid in the liquid. The results thus obtained were concordant and corresponded with the amounts of tartaric acid known to be present. The process is stated to be applicable to wines and other fermented liquors.

According to Kling and Florentin[2] the racemate method for the determination of tartaric acid was found to yield accurate results even in the case of materials containing considerable quantities of iron and aluminium oxides. The results obtained are, in certain cases, somewhat higher than those found by the usual methods, this being due to the fact that the racemate-citrate method gives the whole of the tartaric acid present.

Estimation of Tartaric Acid in the Presence of Malic and Succinic Acids (Wines and Fruit Juices).—The simple evaporation method proposed originally by Pasteur, and modified by Reboul, was found by Mestrezat[3] to give accurate results in the determination of tartaric acid in wines, and is consid-

[1] *Eighth Int. Congress App. Chem.*, 1912, Sect. I, i, 251.
[2] *Ann. Falsif.*, 1912, 5, 518.
[3] *Ann. Chim., Anal.*, 1908, 13, 433.

ered to be a better method than the official (French) process. The author prefers to work as follows: 50 c.c. of the wine are evaporated to the consistency of a syrup; when cold the residue should be semi-fluid, but, if the evaporation has been carried too far, a few drops of water may be added. The basin containing the residue is then placed aside for at least 4 days; after this time, the mass is taken up with a small quantity of 40% alcohol saturated with potassium bitartrate, and the solution is carefully decanted from the crystals. The latter are washed with the same solution, by decantation and then titrated. Results are given showing that concordant analyses may be made by the method and that the presence of malic, succinic and acetic acids, is without influence on the determinations.

Kling and Gobert[1] have applied the racemate method described above to substances which are strongly acid but contain tartaric acid in only small amount, such as vinegar, cider and perry. Fully satisfactory results were obtained, whereas those found by the methods generally used were frequently much too low. It is emphasised that in using the racemate method it is very necessary to use pure l-tartrate and in purifying the commercial product from the d-compound the following procedure has been found convenient: 20 grm. of ammonium l-bitartrate are dissolved in 900 c.c. of water; 20 c.c. of this are diluted to 200 c.c. with water, and to this liquid 10 c.c. of the solution of calcium acetate (supra) added. After half an hour, the precipitate is filtered off, washed, dried and calcined, and from the weight of residue the amount of calcium acetate which should be added to the 880 c.c. to separate all the d-tartaric acid, is calculated. This addition being made, the liquid is allowed to stand for 12 hours, filtered and diluted to a litre, a little formaldehyde being added as preservative. This constitutes the solution b (supra).

Gowing-Scopes[2] has investigated the method proposed by J. von Ferentzy,[3] which is based on the insolubility of basic magnesium tartrate in 50% alcohol and finds that tartaric acid may be accurately estimated in fruit juices by this method, and also in wines, if the tannic acid be first removed. More accurate results are obtained if the basic magnesium tartrate is titrated with permanganate than when it is ignited as recommended by Ferentzy. The following are the details of the process: A solution of the substance under examination, containing not less than 0.05 grm., and not more than 0.10 grm., of tartaric acid, is taken for the estimation; if the bulk of the solution be large, or if alcohol be present, the solution is evaporated to about one-half of the original volume. To the cooled solution is then added an equal volume of absolute alcohol and the precipitate which forms in the case of fruit juices and wines is filtered off and washed with 50% alcohol (by volume). 10 c.c. of ammonia and 10 c.c. of absolute alcohol are added to the filtrate, any precipitate which forms being again removed by filtration and washed with 50% alcohol. To the

[1] *Bull. Assoc. Chim. Sucr.*, 1911, **28**, 760.
[2] *Analyst*, 1908, **33**, 315.
[3] *Chem.-Zeit.*, 1907, **31**, 1118.

filtrate thus obtained are added 10 c.c. of "magnesia mixture" and 10 c.c. of absolute alcohol, the mixture being thoroughly stirred meanwhile. After standing overnight, the solution is filtered through double filter papers, the precipitate is washed with 50% alcohol and is then dissolved off the filter with boiling water, about 400 c.c. being used. The solution is evaporated to a volume of about 200 c.c., or until all the alcohol has been removed, then cooled and diluted to a volume of about 400 c.c., after the addition of 10 c.c. of concentrated sulphuric acid. The solution is next heated to a temperature of 90° C., and potassium permanganate solution is run in a little at a time, until 2 or 3 drops have been added in excess; this excess is then titrated back with oxalic acid solution. The potassium permanganate solution should contain 6.9745 grm. of the salt per litre; 1 c.c. corresponds with 0.0050 grm. of tartaric acid. The oxalic solution should be of equivalent strength.

The following method of estimating malic and tartaric acids in the same solution has been proposed by Dunbar:[1] Solutions of both malic and tartaric acids when treated with uranyl acetate show an increased rotation, which, within certain limits, is proportional to the concentration;[2] both acids reduce definite quantities of potassium permanganate in alkaline solution and form oxalic acid quantitatively. Hence it is possible to calculate the amounts of malic and tartaric acids in a solution, knowing the total change in the rotation on treatment with uranyl acetate, and also either the amount of potassium permanganate reduced or the amount of oxalic acid formed. The details of the method are as follows: 85 c.c. of the solution are rendered slightly alkaline with sodium hydroxide, and 5 grm. of sodium acetate are dissolved in the mixture, which is then made acid with citric acid and diluted to 100 c.c. About 30 c.c. of this solution are shaken for 3 hours with from 2 to 3 grm. of uranyl acetate; if all the latter dissolves more must be added. After filtration, the solution is polarised in a 200 mm. tube, and the reading in degrees Ventzke observed calculated to the basis of the original solution. If optically active substances other than malic and tartaric acids are present, 50 c.c. of the original solution must be treated with an excess of dry powdered lead acetate, filtered, the excess of lead removed from the filtrate by means of anhydrous sodium sulphate, and the solution polarised. The difference between the two readings will give the rotation due to the uranium complexes of the two acids. The quantity of permanganate reduced by the mixed acids is determined by rendering a portion of the solution alkaline with sodium hydroxide, adding an excess of about 3 grm. of the latter and heating the mixture with 50 c.c. of a 1.5% permanganate solution on the water-bath for 1 hour. The mixture is then acidified with sulphuric acid and the excess of permanganate titrated with oxalic acid solution. In the presence of other substances which reduce permanganate but do not form oxalic acid, the amount of the latter may be determined in the usual way by titration with permanganate; 1 c.c. of 1.5% potassium permanganate solu-

[1] U. S. Dept. Agric., Bureau of Chem., Circular No. 105, Oct. 4, 1912.
[2] See J. Soc. Chem. Ind., 1911, 1177, 1407, compare p. 98.

tion is equivalent to 0.0299 grm. of crystallised oxalic acid. Let x be the grm. of malic acid, and y the grm. of tartaric acid, per 100 c.c. of solution, a the polarisation in degrees Ventzke in a 200 mm. tube after treatment with uranyl acetate, and b the grm. of potassium permanganate required to oxidise 100 c.c. of the solution, then $x = -0.0185a + 0.1720b$, and $y = 0.0248a + 0.2436b$. If c equals grm. of oxalic acid yielded by 100 c.c. of the solution then $x = -0.0200a + 0.233c$, and $y = 0.023a + 0.331c$. The data taken into account in these equations are: 1 grm. of malic acid in 100 c.c. will show a rotation of $-27.77°$ V. after treatment with uranyl acetate, and 1 grm. of tartaric acid per 100 c.c. a rotation of $19.61°$ V.; 1 grm. of malic acid reduces 2.8297 grm. $KMnO_4$ and 1 grm. of tartaric acid reduces 2.1062 grm. In the case of fruit juices and solutions containing sugars, 50 c.c. of the solution are mixed with about 150 c.c. of 95% alcohol and the mixed acids are precipitated with lead acetate; the precipitate is collected on a filter, washed with 80% alcohol until free from sugars, then decomposed with dilute sulphuric acid, the lead sulphate is removed by filtration and the alcohol by evaporation and the solution of acids so obtained treated as above described.

Another recent method[1] for the separate estimation of tartaric, malic and succinic acids when occurring together depends on the fact that, under certain defined conditions (faint acidity and relatively low concentration of alcohol), all three acids are precipitated by lanthanum nitrate, whereas other conditions, also closely defined, determine the precipitation of tartaric acid alone, or of tartaric and malic acids. This is the latest result of the work of Dutoit and Duboux referred to on page 13.

Citric Acid.

Citrate of Lime.—The analysis of commercial calcium citrate (citrate of lime) is now generally carried out by Warington's method modified as follows:

4 grm. of the citrate are boiled with 30 c.c. of 2N-hydrochloric acid in a 100 c.c. standard measuring flask for 10 minutes, the solution being then cooled and made up to the mark with water. It is then shaken and filtered through a dry filter paper, 50 c.c. of the filtrate being measured by a standard pipette into a beaker of 300 c.c. capacity and exactly neutralised with dilute sodium hydroxide free from carbonate, using phenolphthalein as indicator. The solution is next made slightly acid by the addition of 3 or 4 drops of N-hydrochloric acid, 2 c.c. of a 45% solution of calcium chloride added, the liquid raised to the boil and kept boiling for 15 minutes; to avoid bumping it is necessary to stir the liquid well until actually boiling, after which it can safely be left. The hot liquid is filtered and the precipitate on the filter paper washed with boiling water 6 times. The filtrate and washings are then made just alkaline by adding a drop or two of dilute ammonia, and boiled down to about 15 c.c. The precipitate which forms is collected on a

[1] P. Dutoit and M. Duboux, *Bull. Soc. Chim.*, 1913 [iv], **13**, 832.

small filter paper and washed with *boiling* water 6 times, using a very small quantity of water for each washing. The filtrate and washings are treated with a drop of ammonia, if they have become acid, and are boiled down to about 10 c.c., but as a rule no further precipitate will be obtained whilst the liquid is hot; any precipitate which forms on cooling can be neglected.

The filter papers with their precipitates are dried at 100° and burnt together in a platinum dish with a cover. The flame should be kept low until the whole is charred, and then gradually raised until the ash is white. The mass is then carefully treated with 30 c.c. of N-hydrochloric acid, and boiled until all is dissolved and all carbon dioxide expelled; the resulting solution is titrated with $N/5$ or $N/2$ sodium hydroxide, using phenolphthalein as indicator.

The sodium hydroxide is standardised by pure potassium hydrogen tartrate, and the $N/1$ hydrochloric acid by the alkali; phenolphthalein is used as indicator.

The number of c.c. of $N/1$ HCl used for the neutralisation of the ash $\times$ 0.070 gives the weight of citric acid in the portion tested.

An almost identical method has been described by L. and J. Gadais.[1]

If the citrate contains much sulphate it is advisable to ash at as low a temperature as possible, preferably with an alcohol flame. Before dissolving in hydrochloric acid, the ash should be treated with 10 c.c. of hydrogen peroxide. [If, as is usual, the hydrogen peroxide contains free acid, allowance must of course be made for it.]

Lime Juice, Lemon Juice, and Factory Citric Acid Liquors.—The analysis of these materials is conducted as follows: 15–20 c.c. of unconcentrated juice, or an amount corresponding with 3 c.c. of concentrated juice (40 grm. per 100 c.c.), previously diluted to facilitate exact measurement, are exactly neutralised with pure potassium hydroxide ($N/5$). The liquid, having a volume of about 50 c.c., is heated to boiling, mixed with a slight excess of concentrated calcium chloride solution, and kept at a gentle boil for half an hour. The precipitate is filtered off immediately while hot, washed with boiling water 6 times, and the mother liquor and washings again evaporated and worked up as described above under calcium citrate. The whole of the calcium citrate collected is then dissolved in 30 c.c. of $N/1$ hydrochloric acid and the excess of acid estimated as above. In dealing with the cruder factory liquors three or four evaporations are generally necessary to separate all the calcium citrate.

The above methods are not entirely free from error,[2] but have not yet been replaced by better. Incorrect results are obtained when the calcium citrate or juice contains other acids which yield sparingly soluble calcium salts. The presence of oxalic acid or of tartaric acid may be detected by the fact that the cold, neutralised solution gives a precipitate in the cold with calcium chloride.

[1] *Bull. Soc. Chim.*, 1909 [iv], **5**, 287.
[2] *Cf.* O. von Spindler, *Chem. Zeit.*, 1903, **27**, 1263.

Other Methods of Estimation.—The above-described methods are those which are generally adopted in the citric acid industry. The following
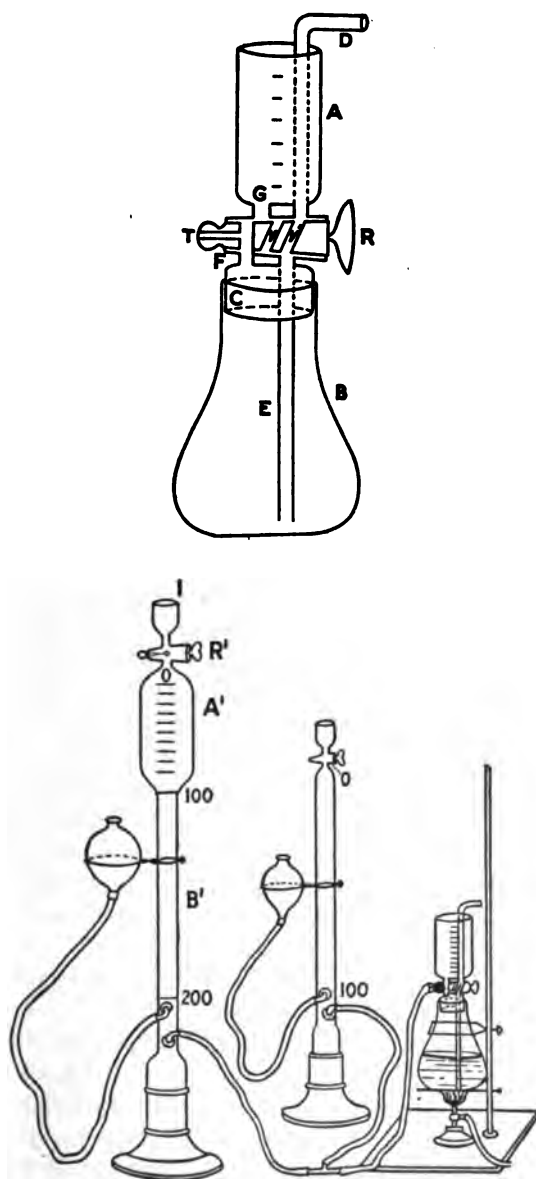
FIG. 5.

methods, selected from the numerous processes which have been suggested may be here briefly summarised.

C. Ulpiani and A. Parozzani[1] have described a method of analysis which, according to Klapproth (Lunge-Keane, Vol. III,) gives satisfactory results for citric acid even in presence of other organic acids. This method depends upon the fact that citric acid, in presence of a sufficient quantity of calcium chloride, is precipitated by sodium hydroxide *in the cold*, when the whole of the acid is saturated, and in the *hot* solution when one-third of the acid is saturated.

Spica[2] has described a method based on the formation of carbon monoxide when citric acid is gently warmed with concentrated sulphuric acid; 1 mol. of carbon monoxide is obtained for each mol. of citric acid present. The citric acid is first precipitated as calcium citrate (as in Warington's method) and the citrate then decomposed by the sulphuric acid in the apparatus described below (Fig. 5):

The upper part A, is fitted to the flask, B (150 c.c. capacity), by a ground joint, and the tubes, D and E and C and E, may be connected respectively through the tap, R, as also may the flask and the exterior. 2 grm. of the calcium citrate, moistened with water, are introduced into B, and the air in the flask is completely displaced by carbon dioxide, the absence of air being ascertained by means of an auxiliary nitrometer, filled with potassium hydroxide solution (1 : 5) and attached to the T-piece. 25 c.c. of concentrated sulphuric acid are then run into B from A, and a slow current of carbon dioxide is again passed into the flask, which is warmed to 80°–100° C., and occasionally shaken, the carbon monoxide evolved being collected in a nitrometer of 200 c.c. capacity, of which the lower part B[1] (100 c.c. capacity) is graduated in fifths of a cubic centimetre. When the volume of gas becomes constant, the nitrometer is allowed to stand for half an hour and then, after washing the gas with potassium hydroxide solution, introduced through I, the volume is read and the usual corrections are made for temperature and pressure. 1 c.c. of carbon monoxide at 0° C. and 760 mm. indicates 0.009407 grm. of citric acid ($C_6H_8O_7,H_2O$). The same apparatus may be used for the determination of carbonate in a citrate, by decomposing with a known volume of concentrated hydrochloric acid and measuring the evolved carbon dioxide over water.

The above process is obviously useless when other organic acids are present which evolve carbon monoxide.

Estimation of Citric Acid in Presence of Other Acids.—Beau's modification of the method of Denigès (*Ann. Chim. Phys.*, 1899, 18) is shown by Gowing-Scopes[3] to give unreliable results owing to the action of the hydrogen peroxide (added to remove the manganese dioxide) upon the precipitated mercury dicarboxysulphoacetone. Other reagents tried for removing the manganese dioxide either oxidised or reduced the precipitate, which in hot solutions was also oxidised by manganese dioxide. A compound containing

[1] *Atti. R. Accad. Lincei*, 1906 [v], 15, ii, 517.
[2] *Chem. Zeit.*, 1910, 34, 1141; compare Barboni, *Ann. Lab. Centr. delle Gabelle*, 1912, p. 311.
[3] *Analyst*, 1913, 38, 12.

72.5 to 74.0% of mercury and apparently similar to, if not identical with the compound of Denigès was obtained by the use of a reagent prepared by adding 68 c.c. of strong nitric acid to 51 grm. of mercuric nitrate and 51 grm. of manganese nitrate, diluting the mixture with 100 c.c. of water, and finally, making up the solution to 250 c.c. and filtering. For the determination of citric acid a quantity of the substance containing not more than 0.04 grm. nor less than 0.001 grm. of the acid is exactly neutralised with $N/10$ alkali, using phenolphthaleïn as indicator, and, after the addition of 10 c.c. of the reagent, the liquid is diluted to 200 c.c., and boiled for 3 hours beneath a reflux condenser. The precipitate is washed by decantation, collected on a weighed Gooch crucible, and again washed in the crucible, which is then dried in the water-oven until nearly constant in weight (about 5 hours). The residue should then be of a cream colour, any yellow colouration indicating the formation of basic salts, which will cause the results to be too high. One-sixth of the weight of the precipitate gives the amount of citric acid. In 16 test estimations with pure citric acid within the limits stated above, the maximum error was +0.0003 and −0.0004 grm. Good results were also obtained in the presence of tartaric, succinic, oxalic, benzoic, and phosphoric acids, but when malic, lactic or salicylic acids were present the results were too high. Salicylic acid gives, with the reagent, a salmon-coloured precipitate, probably a nitro-derivative, whilst gallotannic acid gives an orange-brown precipitate. The precipitate given by citric acid decomposes suddenly when heated. It is very soluble in hydrochloric acid, in strong sulphuric or nitric acid, and in solutions of halogen salts.

In the absence of sugars it is not necessary to precipitate the citric acid as barium citrate and redissolve with phosphoric acid. The author claims an accuracy of 2% to 4%.

The above process does not give satisfactory results in presence of malic and tartaric acid and is therefore not applicable to the examination of fruit juices. In such cases, according to D. S. Pratt[1] the following method is of especial value.

50 grm. of fruit juice are treated with 110 c.c. of 95% alcohol to remove pectin bodies. After 15 minutes the solution is filtered and the residue washed with 95% alcohol. The resulting solution is diluted with water to an alcoholic strength of about 50%, and a 20% aqueous solution of barium acetate is added to precipitate the citric acid. After stirring, allowing the precipitate to settle, and filtering the solution, the precipitate on the paper is washed with 50% alcohol to remove sugars and then the paper and its contents are dried to remove the alcohol. The residue is then warmed with 50 c.c. of water and 3–5 c.c. of syrupy phosphoric acid to dissolve the barium citrate. This mixture is filtered into a graduated flask and the paper washed until the filtrate measures 100 c.c. An aliquot part of this solution containing 0.05–0.15 grm. citric acid is measured into a 500 c.c. dis-

[1] *U. S. Dept. Agr. Bur. Chem.*, Circular **88** (1912).

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tilling flask, 5–10 c.c. phosphoric acid are added with 400 c.c. hot water and the flask is heated. When briskly boiling 0.05% potassium permanganate solution is run in by means of a dropping funnel at the rate of 1 to 2 drops per second until the pink colouration is permanent.

The acetone formed by the oxidation distils off as fast as it is formed into 30–40 c.c. of Denigès[1] reagent. The distillation is continued till only 50–100 c.c. of solution remain.in the flask.

The mixture in the receiver is then boiled gently under a reflux condenser for 45 minutes after the liquid becomes cloudy. It is then filtered hot through a Gooch crucible, washed with water, alcohol, and ether and dired in a water-oven for 30 minutes. The weight of precipitate multiplied by 0.22 gives the weight of citric acid originally present.

W. Fresenius and L. Grünhut[2] claim that the methods based on the precipitation of acetone dicarboxylic acid as given above are untrustworthy in the qualitative detection of citric acid in wines. For this purpose they recommend Krug's modification of Moslinger's test which is carried out as follows:

50 c.c. of wine are evaporated to a syrup. The syrup is treated with 95% alcohol and filtered to remove tartrates and then evaporated to remove alcohol. 10 c.c. of the resulting liquid are treated with acetic acid and lead acetate. In the presence of citric acid a precipitate will be obtained which dissolves on heating and reappears on cooling.

For recent data as to the composition of grape juices and notes on the determination of their acidity, see Gore.[3]

Tests of Purity of Citric Acid, Lead, Arsenic, and Ash are tested for as under tartaric acid, the limiting quantities allowed being the same as in the case of tartaric acid (see page 101). It is usual, however, in commerce to require a higher degree of freedom from lead than in the case of tartaric acid; the amount present seldom exceeds 10 parts per million. Arsenic is generally entirely absent.

British Pharmacopœia 1914.—The following are the requirements: 1 grm. dissolved in water requires for neutralisation 14.2 c.c. of $N/1$ solution of sodium hydroxide; yields no characteristic reaction for copper or iron and not more than very slight reaction for calcium or sulphates. *Lead limit* 20 parts per million. *Arsenic limit* 1.4 parts per million. 1 grm. of powdered citric acid mixed with 10 c.c. of sulphuric acid in a test-tube previously rinsed with sulphuric acid acquires not more than a pale yellow colour when kept at a temperature of 90° for 1 hour (absence of tartaric acid); ash not more than 0.05%.

The following colour tests, based on Pusch's method of detecting tartaric acid in citric acid are described by Hill.[4] 0.5 grm. of the sample and of pure

[1] Denigès reagent is made by adding 200 c.c. of concentrated sulphuric acid with constant stirring to a suspension of 50 grm. of mercuric oxide in 500 c.c. of water. This mixture is heated on a water-bath to ensure complete solution. It is then filtered, cooled and diluted to 1,000 c.c.
[2] *Zeit. anal. Chem.*, 1913, 52, 31.
[3] *J. Ind. Eng. Chem.*, 1909, 1, July.
[4] *Pharm. J.*, 1910, 84, 245.

citric acid are placed in separate test-tubes (6 in. $\times$ ⅝ in.) and 5 c.c. of sulphuric acid added to each. The tubes are placed simultaneously in an ordinary Bunsen flame; at the end of 30 seconds they are withdrawn and examined. The results obtained with acids of different degrees of purity are as follows:

Pure acid	= lemon-yellow solution.
5.0% of tartaric acid	= black, sulphur dioxide abundant.
1.0% tartaric acid	= deep brown-black, sulphur dioxide evident.
0.5% tartaric acid	= deep red-brown.
0.25% tartaric acid	= red-brown.
0.1% tartaric acid	= reddish-brown.
0.01% tartaric acid	= brownish-yellow.

With careful manipulation and 60 seconds heating, 0.0001% of tartaric acid is said to be easily detected; even 0.00001% gave a distinctly deeper tint, in good light, than the control tube. The test is also applicable to citrates, 1% of tartrate being easily detected. The above method is also applicable to detect sugar in citric acid; the reactions obtained are:

1% of sugar	= cherry-red, sulphur dioxide distinct.
0.1% of sugar	= sherry colour, sulphur dioxide evident.
0.01% of sugar	= yellowish-red, sulphur dioxide perceptible.
0.001% of sugar	= reddish-yellow.
0.0001% of sugar	= reddish-yellow.

It was also found that 0.5% of sugar could be detected, in tartaric acid, by the colouration after an hour's contact with cold sulphuric acid; by heating for 10 seconds in the flame the control tube remained practically unaffected, with 1% of sugar a red solution was obtained, and with 0.5% a red-brown ring.

Häussler[1] describes a characteristic colour reaction given by citric acid with vanillin; the solution is evaporated to dryness after adding an alcoholic solution of vanillin and the residue treated with 3 drops of dilute sulphuric acid, heated on a water-bath for 15 mins., dissolved in water and ammonia added. A bright-red colouration is obtained with 0.002 grm. of citric acid. The red colouration is not given by tartaric, malic, oxalic, malonic, benzoic, salicylic, acetic, lactic or succinic acids.

[1] *Chem. Zeit.*, 1914. 38, 937.

Page 546, line 4 for "flask" read "beaker;" line 8, for "10 c.c." read "100 c.c.;" line 10 from bottom, for "Warrington" read "Warington."

Page 549 line 13, for "dissolved" read "treated."

Page 555, line 10, "Good lemons yield" should read "Good lemon juice yields."

Page 560, In the table at top of page, "Lime juice" "Lemon juice" should be interchanged.

Page 566, line 14 from bottom, "substracting" should read "subtracting;" line 13 from bottom, "form" should read "former;" line 8 from bottom, "hydroade" should read "hydroxide."

Page 573, in Index, insert "Gallisin," 304, 379. Delete "Læonlose," and insert after "Lævulose," p. 373.

Page 574, insert "Maltase," p. 361.

FIXED OILS, FATS AND WAXES.

By C. AINSWORTH MITCHELL.

EXTRACTION OF FAT.

Grimme[1] has made comparative estimations in which fat was extracted with various chlorohydrocarbons and with ether. The following average percentage results were obtained:

Ether	CHCl₃	CCl₄	C₂H₂Cl₂	C₂HCl₃	C₂Cl₄	C₂H₂Cl₄	C₂HCl₅
7.45	8.58	7.43	8.13	7.46	7.79	7.71	9.62

Only in the case of carbon tetrachloride and trichlorethylene did the results agree with those obtained with ether.

Complete extraction of the fat from cottonseed was not obtained with less than 100 c.c. of either cold solvent, but 45 minutes' extraction was sufficient with carbon tetrachloride and 30 minutes' with trichlorethylene.

With proportions below 10% of fat the weight of the residue left on evaporating 50 c.c. of the extract may be accepted as sufficiently accurate, but an addition of 0.2% should be made for amounts between 10 and 15%, and of 0.4% for amounts between 15 and 20%. Preliminary drying of the material was found by Grimme to be unnecessary.

Gowing-Scopes[2] confirms the suitability of cold trichlorethylene for the extraction of fat, but points out that it is advisable to dry the solvent to prevent the formation of the hydrochloric acid, which would act upon the fat.

He recommends for the extraction a modification of the apparatus devised by Beadle and Stevens[3]

The physical properties of the solvents are shown in the following table of Gowing-Scopes.[4]

Chlorohydrocarbon	Boiling point, °C.	Freezing point, °C.	Sp. gr. at 25°C.	Coefficient of expansion	Heat of vaporisation, calories	Refractive index	Viscosity at 25° C.	Specific heat
Chloroform..........	61.5	......	1.4791	0.001257	....	1.449 (15°C.)	.....	.....
Carbon tetrachloride..	76.7	......	1.5835	0.001227	....	1.464 (15°C.)	.....	.....
Dichlorethylene, Cis....	48.8 (at 763 mm.)	......	1.3328	0.001360	6930	....	0.457	.....
Dichlorethylene, trans.	59.8 (at 763 mm.)	......	1.3545	0.001270	7268	....	0.510	.....
Trichlorethylene......	87.5	− 73	1.4542	0.001193	7436	1.47914 (17°C.)	0.615	0.223
Tetrachlorethylene....	121.0	− 19	1.6080	0.001078	8554	....	0.940	0.216
Tetrachorethane......	147.2	− 36	1.5881	0.000998	9134	1.49559 (17°C.)	1.808	0.268
Pentachlorethane.....	159.1	− 22	1.6712	0.000909	8829	....	2.432	0.266
Hexachlorethane......	185.5 (at 776.7 mm.)	−187	2.01(?)	....	....	....	.....	.....

[1] *Chem. Rev. Fett. Ind.*, 1912, 19, 191.
[2] *Analyst*, 1914, 39, 4.
[3] *Analyst*, 1913, 38, 143.
[4] *Analyst*, 1914, 39, 5.

Constitution and Chemical Properties.

Theory of Saponification.—Experiments in which triolein was fractionally saponified with alcoholic alkali have led Fortini[1] to the conclusion that saponification takes place in three distinct phases. The acetyl values of the fractions also supported the views of Lewkowitsch.

Unsaponifiable Matter.

Detection of Phytosterol and Cholesterol.—Marcusson and Schilling[2] devised a method of separating phytosterol or cholesterol by precipitation with digitonin. A simple modification of the method is recommened by Fritzsche. 50 grm. of the melted fat are mechanically stirred for 5 minutes at 60° to 70° C. with 20 c.c. of a 1% alchoholic solution of digitonin. In the case of fluid and semi-solid fats the mass is at once filtered, with the aid of suction, in a Buchner's funnel, and the residue washed 6 times with ether (5 c.c. each time); in the case of solid fats 20 c.c. of chloroform are added to the hot liquid and the residue washed with two portions (4 c.c.) of hot chloroform and then with six portions of ether to remove all fat. The residue (digitonide) is dried for about 5 minutes at 30° to 40° C., dissolved in 2 c.c. of hot acetic acid, and the solution boiled for about 5 minutes in a test-tube with a vertical tube to act as condenser and then filtered through cotton wool. The tube and filter are twice washed with 0.5 c.c. of hot absolute alcohol, and the combined filtrate and washings evaporated on the water-bath in a current of air. The residual phytosteryl or cholesteryl acetate is dissolved in 0.5 to 1 c.c. of absolute alcohol and the crystals drained on porous porcelain and examined in the usual way (see also section on *Butter*).

Alcoholysis.—The method has been applied to the examination of castor and linseed oils by Haller[3] and of japan wax by Tassily.[4]

Elsdon[5] has submitted the process to critical examination and shows that although it determines the nature of the fatty acids contained in a fat, it is too tedious for ordinary laboratory work, and is only roughly quantitative. The results obtained may show the relative amounts of the constituents, but are probably not within 5 to 10% of the true values.

Bromine and Iodine Absorptions.

Insoluble Bromide Test.—(See also under *Linseed Oil*, page 189.) Sutcliffe[6] recommends the following modification as giving results agreeing to within about 1%: 1 grm. of the oil is dissolved in 40 c.c. of ether in a weighed flask, 5 c.c. of glacial acetic acid are added, and the flask and its

[1] *Chem. Zeit.*, 1912, 36, 1117.
[2] *Chem. Zeit.*, 1913, 37, 1901.
[3] *J. Soc. Chem. Ind.*, 1907, 26, 328; 1908. 27, 234.
[4] *Ibid.*, 1911, 30, 907.
[5] *Analyst*, 1913, 38, 8.
[6] *Analyst*, 1914, 39, 28, 338.

contents cooled in water to about 11°. Bromine is then added drop by drop until the solution is red, and the flask is closed and allowed to stand for 12 hours in water. The contents are filtered through a disc of paper in a Gooch crucible and the precipitate washed 3 times by decantation and twice in the crucible with 10 c.c. of ether chilled to 5°; it is dried for 3 hours in the water oven, and weighed. Its melting point should range from 141° to 144° in the case of linseed oil.

Comparative determinations of the amounts of insoluble bromide and of the iodine value of linseed oils of various origin, showed that under these conditions the relationship between the two values could be expressed by the formula: Per cent. of bromide = (0.63 iodine value) −78.0. The precipitated bromides must be white and crumble readily when dried. Certain sorts of bromine give products which when dried are dark and horny; these should be rejected.

Gemmell[1] was unable to obtain concordant results with various methods, mainly owing to the solubility of the bromides in the various solvents and he therefore recommends the following method, applied to the fatty acids, as being preferable to estimating the bromide obtained directly from the oils: The oil (5 grm.) is saponified with alcoholic potassium hydroxide, the soap dried and dissolved in 100 c.c. water, and the fatty acids liberated in the usual way. The flask is cooled in water, and the fatty acids separated by shaking with ether. The united ether extract (100 c.c.) is divided into aliquot portions (20 c.c.), and to each of these is added 2 c.c. of glacial acetic acid. The flasks are chilled in ice-water and the solution brominated and allowed to stand, the liquid decanted, the precipitates washed thrice in the flasks with chilled ether, then transferred (by means of 5 c.c. of ether) to a weighed filter paper, dried and weighed.

The following results were thus obtained: Raw linseed oils (7 kinds) 32.60 to 37.65; boiled linseed oils (6 samples), 25.95 to 33.90; soja bean oil, 4.10; rape oil, 2.35; and walnut oil, 3.0%; china wood oil, *nil*. Satisfactory results may also be obtained in the way with marine animal oils, as is shown by the following typical examples: Cod-liver oil, 35.20; whale oil, 21.70; brown whale oil, 25.80; menhaden oil, 51.70; shark-liver oil, 17.70; and sperm oil, 1.70.

The main objection to Gemmell's modification is the risk of loss of linolenic acid by oxidation during the liberation of the fatty acids. Some of his criticisms upon the direct methods have been answered by Sutcliffe.[2]

The loss due to solubility might possibly be overcome, at all events in the case of linseed oil, by previously saturating the solvent with the insoluble bromide purified by extraction with ether.

Gemmell (*loc. cit.*) points out that the solubility of the bromide from marine animal oils is less than that of the bromide from vegetable oils, so

[1] *Analyst,* 1914, 39, 297.
[2] *Analyst,* 1914, 39, 388.

much so that a precipitate is formed as soon as bromine is added. He suggests that this affords a rapid means of detecting fish oils in vegetable oils.

In his opinion the insoluble bromide formed by linseed oil is not that of a mixed glyceride, but the reasons given in support of this view are not conclusive.

Stiepel has found[1] that the amount of insoluble bromide from linseed oil is greatly reduced by heating the oil, while the analogous bromide obtained from marine animal oil is no longer formed after heating. Hence a negative result of the "octobromide" test cannot of itself be regarded as a proof of the absence of marine animal oil.

Thus the commercial product *neutraline*, which consists of deodorised fish oil, yields no insoluble bromide, and might therefore be taken for an animal hoof oil.

Iodine Value.—Comparative determinations of the iodine values of erucic, elaidic, oleic, ricinoleic and undecylic acids, made by Weiser and Donath[2] by the methods of Hübl, Waller, Wijs and Winkler, gave practically concordant results. In the case of linoleic acid the only method that gave theoretical results was that of Winkler, the other methods giving too high values. The iodine values of crotonic, tiglic and cinnamic acids could not be determined by the methods of Hübl, Waller and Wijs, whereas nearly theoretical results were obtained by Winkler's method.

Winkler's Method.[3]—From 0.1 to 0.5 grm. of the fat is dissolved in 10 c.c. of carbon tetrachloride, and the solution treated with 50 c.c. of Winkler's solution ($N/10$ potassium bromate solution containing 1 to 1.5 grm. of potassium bromide and 10 c.c. of 10% hydrochloric acid). After 30 minutes to 2 hours (according to the degree of unsaturation of the fat) 10 c.c. of 10% potassium iodide solution are added and the liberated iodine titrated with thiosulphate solution.

Meigen and Winogradoff[4] show that unsaturated fatty acids (oleic acid) absorb more chlorine than iodine from a mixture of the 2 halogens, while more or less substitution of the chlorine occurs. This substitution is checked by the presence of acid. It is inadvisable, however, to add a large excess of hydrochloric acid (as in Waller's solution) since combination of oleic acid with the hydrochloric acid will then take place. In examining an unknown compound Meigen and Winogradoff advocate the use of Wijs' method, with the addition that after the titration with thiosulphate the product of the action is extracted with water, and the amount of halogen acid in the aqueous extract is titrated with $N/10$ alkali.

Thus a sample of pure oleic acid treated for 30 minutes with a Wijs' solution containing 13 grm. of iodine per 1,000 c.c. and an equivalent quantity of chlorine gave an iodine value of 99.95 (theory, 89.95), while the acid

[1] *Chem. Zent.*, 1912, 2, 175.
[2] *Zeit. Untersuch. Nahr. Genussm.*, 1914, 28, 65.
[3] *Pharmacop. Hungarica*, 1900, XI.
[4] *Zeit. angew. Chem.*, 1913, 27, 241.

in the aqueous solution corresponded to 4.62% of substituted iodine. With a Wijs' solution containing an excess of 2% of iodine over the chlorine the iodine value found was 90.95, while the acid in the aqueous extract corresponded to 0.53% of substituted iodine. When there was an excess of 10% of chlorine in the Wijs' solution the iodine value of the oleic acid was 105.40, and the acid in the aqueous solution corresponded to 8.31% of iodine. It was proved that halogen acids were only formed by substitution.

Gowing-Scopes[1] studied the effect of using different chlorohydrocarbons as solvent in Wijs' method and found that the results obtained with trichlorethylene, tetrachlorethylene, tetrachlorethane and pentachlorethane agreed closely with those obtained with carbon tetrachloride, but that the figures with dichlorethylene were too low.

Acetyl Value.—To obtain results comparable with the other values of fat analysis Holland[2] suggests that the acetyl value should indicate the number of milligrams of potassium hydroxide required to saponify the acetyl taken up by 1 grm. of the fat on acetylation.

It may be rapidly determined as follows: 5 grm. of the fat are heated with 10 c.c. of acetic anhydride over boiling water beneath a reflux condenser for 1½ hours, sufficient ceresin to form a solid disc when cold being then introduced. After the addition of 150 c.c. of boiling water the flask is again heated on the water-bath, occasionally shaking, to expel acetic acid and then cooled. The solid cake is again heated with 150 c.c. of boiling water, and this process repeated about 6 times, until the filtrate is nearly neutral.

The solid disc and particles on the filter are now boiled with 50 c.c. of standard alcoholic potassium hydroxide solution and 50 c.c. of alcohol, beneath a reflux condenser (with glass beads to prevent bumping) and the excess of alkali titrated with standard hydrochloric acid.

The difference between the saponification values before and after acetylation is the acetyl value.

Oxidation of Oils—Drying Properties.

Gravimetric Estimation of Absorption of Oxygen during Drying.—A method devised by Krumbhaar[3] of measuring the amount of oxygen absorbed by oils during drying may also afford a means of distinguishing between different drying oils. A weighed quantity of the oil is mixed with 0.6% of cobalt resinate and spread over filter paper, which is placed in a U-tube immersed in water at 30° C. A steady current of dry air (free from carbon dioxide) is drawn through this tube, and the volatile products formed in the drying process are passed, first through a strongly heated tube of copper oxide (to complete their decomposition into carbon dioxide and water) and

[1] *Analyst*, 1914, 39, 19.
[2] *J. Ind. Eng. Chem.*, 1914, 6, 482.
[3] *Chem. Rev. Fett. Ind.*, 1913, 20, 290.

then through weighed absorption tubes containing calcium chloride and soda-lime. After every 2 hours the air in the apparatus is replaced by nitrogen, the taps closed, and the tube containing the oil and the absorption tubes weighed, this being continued until the weight becomes constant. The sum of the increase of weight in the oil tube, of the hydrogen absorbed by the calcium chloride tube and of the carbon absorbed by the soda-lime tube gives the amount of oxygen taken up by the oil.

For example, 0.743 grm. of linseed oil showed an increase in weight of 0.128 grm. in 18 hours, while the amounts of hydrogen and carbon volatilised were 0.009 and 0.016 grm. respectively, giving a total of 0.153 grm. or 20.6% of oxygen absorbed by the oil. (See also under Linseed Oil, page 193.)

Colour Tests of Oils.

Colour indications given by oils with certain phenols in the presence of nitric acid are described by Malacarne.[1] The most distinctive is the violet colouration given by sesame oil with resorcinol or phloroglucinol.

Catalytic Hydrogenation of Oils—Hardened Oils.

During the last 3 years the analytical problems in the examination of fats have been greatly complicated by the general introduction of hydrogenated oils as commercial products.

Theoretically it should be possible to convert oleic, linoleic and other unsaturated fatty acids and glycerides into the corresponding solid acids of the stearic series by the simple addition of hydrogen, the process being analogous to the absorption of halogens or oxygen by the unsaturated compounds

$$C_{18}H_{34}O_2 + H_2 = C_{18}H_{36}O_2$$

Prior to 1902, however, all attempts to hydrogenate oils by this method proved unsuccessful, but in that year Le Prince and Siveke (Germ. Pat. 141029) claimed a process of solidifying oils by heating them with hydrogen in the presence of a catalytic agent; and an analogous English patent (No. 1515 of 1905) was taken out by Normann.

The development of the new industry and the types of apparatus used in the various processes are described by Ellis.[2] See also Crossley.[3]

The catalytic agents most commonly used in the commercial processes are nickel and its salts, and palladium salts, which are usually precipitated in a fine state of division over a porous material such as pumice stone, or kieselguhr. Other catalysts include cobalt, iron, platinum, and osmium, and their oxides and other salts. The presence of traces of the catalytic

[1] *Chem. Zentr.*, 1913, **1**, 2183.
[2] *J. Soc. Chem. Ind.*, 1912, **31**, 1155.
[3] *J. Soc. Chem. Ind.*, 1914, **33**, 1135.

agent, especially nickel, in the hardened fats sometimes affords a proof of the origin of the material.

Commercial Hardened Oils.—Speaking generally the solidified products obtained by hydrogenating whale and fish oils are only used for technical purposes, such as soap-making. Examples of such fats are *talgol* and *candelite*, which are made at Emmerich.

Edible hardened oils, prepared from cottonseed, sesame and other oils are being increasingly used in Europe and America in the preparation of margarine and lard substitutes. One of the best known German edible hardened oils is sold under the name of *brebesol*. The physical condition of the products ranges from a semi-solid mass resembling butter to a hard tallow, according to the degree of hydrogenation of the oil.

Analytical Constants of Hardened Oils.—(See also under Margarine page 173). Hydrogenation of an oil lowers its refractometer reading and iodine value, and raises its melting point, but has little effect upon the saponification value.

The following results were obtained by Bömer and Leschly-Hansen[1] in the examination of oils hardened by heating in an autoclave in a current of hydrogen in the presence of nickel reduced on kieselguhr.

Oil	M. p. °C.	Solid. pt. °C.	Refractometer reading at 40° C.	Acid value	Sapon. value	Iodine value
Arachis	51.2	36.5	50.1	1.0	188.7	47.4
Sesame	47.8	33.4	51.5	0.5	190.6	54.8
Sesame, technical	62.1	45.3	38.4 (at 50°C.)	4.7	188.9	25.4
Cottonseed	38.5	25.4	53.8	0.6	195.7	69.7
Whale	45.1	33.9	49.1	1.2	192.3	45.2
Coconut, natural	25.6	20.4	37.4	0.3	255.6	11.8
Coconut, hardened	44.5	27.7	35.9	0.4	254.1	1.0

The liquid fatty acids showed the following iodine values: Hardened arachis oil, 82.9 to 91.8; sesame oil, 88.9; cottonseed oil, 115.6; and whale oil 96.0.

The reduction in the refractive index caused by hydrogenation is shown by the following examples given by Ellis:[2] Maize oil, 1.4514; whale, 1.4550; soja bean, 1.4538; coconut "olein," 1.4425; linseed 1.4610; palm, 1.4517; and arachis oil, 1.4547.

Hardened marine animal oils are deodorised in the process, and acquire the appearance of tallow. Two samples analysed by Grimme[3] gave the following values:

Sp. gr. at 15° C.	M. p °C.	Solid. pt. °C.	Refractive index at 40° C.	Acid value	Sapon. value	Iodine value (Wijs)
0.9271	47.2	34.9	1.4529	1.94	189.3	23.24
0.9256	38.5	31.5	1.4575	1.00	188.8	58.34

[1] *Chem. Rev. Fett.Ind.*, 1912, 19, 218, 247.
[2] *J. Ind. Eng. Chem.*, 1914, 6, 117.
[3] *Chem. Rev. Fett. Ind.*, 1913, 20, 129.

Colour Tests.—Hardened marine animal oils often give intense colourations with concentrated mineral acids, but these do not agree with colourations described as characteristic of the untreated oils. The intensity of the colouration varies with the degree of hydrogenation. Sulphuric acid containing a trace of iodine gives a violet-red colouration with hardened whale and fish oils.

Bellier's reagent for seed oils (nitric acid, sp. gr. 1.4 and resorcinol in benzene) gives somewhat different shades of colour with hardened sesame, arachis and cottonseed oils than in the case of the original oils. With hardened marine animal oils both acid and oil give an orange-yellow colouration.[1]

Arachidic acid may be detected in hardened arachis oil (*q.v.*) but a suitable modification is required.

The Baudouin test for sesame oil is intensified, whereas Haphen's cottonseed oil test is inhibited. Hauchehorne's test for cottonseed oil (*q.v.*) is not affected.

According to Leimdörfer[2] the stearic acid formed in the hydrogenation of oils is chemically identical with natural stearic acid, but the stearin of hydrogenated oils differs in crystalline character and other physical properties from the stearin of ordinary fats.

Hydroxyl groups are more or less split off in the hydrogenating process. Thus the hydroxyl value of a sample of castor oil fell from 156 to 102.[3]

The proportion of insoluble bromides given by linseed and marine animal oils is greatly reduced by hydrogenation, so that the insoluble bromide test will not give the same result as before and may even fail to detect the presence of these oils.

Unsaponifiable Matter.—Hydrogenation also reduces the amount of cholesterol or phytosterol in the oil, and in proportion to the degree of hardening. The process affects cholesterol more than phytosterol. Thus it has been found by Marcusson and Meyerheim[4] that 75% of cholesterol was resinified during hydrogenation at 200°, whilst phytosterol was not appreciably affected. After treatment at 250° cholesterol no longer gave any crystalline derivatives.

This explains why cholesterol cannot be isolated from *talgol* and similar hardened products of animal oils.

Detection of Nickel in Hardened Oils.—Bömer and Leschly-Hansen (*loc. cit.*) recommend the dimethylglyoxime test: From 5 to 10 grm. of the fat are mixed with strong hydrochloric acid in a test-tube which is immersed with frequent shaking for 30 minutes in boiling water. The acid extract (filtered if necessary through animal charcoal) is then evaporated and the residue tested with a 1% alcoholic solution of dimethylglyoxime.

Bömer[5] found 0.01% of ash with 0.006% of nickel oxide in hydrogenated

[1] Kreis and Roth: *Z. Untersuch. Nahr. Genussm.*, 1913, **25**, 81.
[2] *Chem. Zentralbl.*, 1914, **I**, 304.
[3] Normann and Hugel: *Chem. Zeit.*, 1913, **37**, 815.
[4] *Zeit. angew. Chem.*, 1914, **27**, 201.
[5] *Chem. Rev. Fett Ind.*, 1912, **19**, 221.

sesame oil and 0.006% of ash with 0.0045% of nickel oxide in hardened whale oil.

The physiological significance of traces of nickel in hardened oils is discussed by Ellis,[1] Knapp,[2] Bömer (*loc. cit.*); and Offerdahl-Larvik.[3]

It has been found by Prall that in some cases pure untreated oils may give a red colouration; while Kerr[4] has shown that hydrogenated cottonseed oil free from nickel may yield to hot hydrochloric acid an organic base, which will give with dimethylglyoxime and ammonia a red colouration, closely resembling that obtained with traces of nickel except that it is fugitive. Hence before applying the test for nickel the residue should be treated with 2 to 3 c.c. of nitric acid to destroy organic matter. Owing to this uncertainty, Knapp[5] prefers the less sensitive ammonium sulphide test: 50 grm. of the fat are heated with 20 c.c. of hydrochloric acid with vigorous shaking, the acid extract is evaporated to dryness, and the residue dissolved in 1 drop of water, and tested on a white tile with 1 drop of ammonium sulphide (compare also page 173).

[1] *J. Soc. Chem. Ind.*, 1912, 31, 1166.
[2] *Analyst*, 1913, 38, 102.
[3] *Ber. deutsch. Pharm. Ges.*, 1913, 23, 558.
[4] *J. Ind. Eng. Chem.*, 1914, 6, 207.
[5] *Analyst*, 1913, 38, 103.

SPECIAL CHARACTERS AND MODES OF EXAMINING FATS, OILS AND WAXES.

By E. R. BOLTON and CECIL REVIS.

ARACHIS OIL.[1]

The Indian or Mozambique nuts are usually decorticated before shipment to Europe. As they undergo "heating" on the voyage they cannot be used to produce the best edible oil and are mainly worked up for soap oils. The Bombay nuts yield a somewhat better quality and the largest nuts are those cultivated in Fiji. The greatest quantity of Arachis nuts comes from Senegal.

Bellier's test has been very carefully investigated by Evers[2] who has confirmed the statement made in Vol. II, that low results are obtained by this process as usually carried out.

He has compared the method with Renard's process and obtained the following results:

TABLE I.

Oil	Renard			Bellier		
	Arachidic acid, %	M. p., °C.	Arachis oil, %	Arachidic acid, %	M. p., °C.	Arachis oil, %
Arachis (A)	4.59	73.5	....	3.56	71	78
Arachis (B)	5.15	72.0	....	3.76	71	83
Olive oil, Nice superfine	0	....	....	0	....	....
Olive oil, "seconds"	0	....	....	Trace	....	....
Olive oil, Malaga	0	....	....	Trace	....	....
Olive oil, 50% } Arachis (A), 50% }	2.28	73.5	50	1.36	72	30

Evers suggests that the low results obtained may be due either to the solubility of arachidic and lignoceric acids in 70% alcohol or to the incomplete precipitation of the fatty acids on account of their solubility in the strong solution of oleic and other fatty acids. Renard[3] states that arachidic and lignoceric acids are quite insoluble in 70% alcohol, but Evers contests this statement and having prepared these acids from arachis oil by Renard's process, obtained the following mean values for their solubility in 70% alcohol under the stated conditions.

[1] Lewkowitsch, *Chem. Tech. Oils and Fats*, Ed. v, 2, 298.
[2] *Analyst*, 1912, 37, 487.
[3] *Compt. rend.*, 73, 1330.

About 0.2 grm. fatty acid was dissolved in 93% alcohol and sufficient water added to reduce the strength of alcohol to 70%. After standing for several hours the liquid was filtered and a measured volume evaporated to dryness, the solubilities given below being calculated from the weight of residue.

TABLE II.

Melting point	Solubility, grams per 100 c.c.	
	At 13° C.	At 18° C.
71° C.	0.016	0.023
72° C.	0.012	0.017
73° C.	0.009	0.012

The solubility was also determined when the fatty acids were washed on a filter paper, about 0.2 grm. being used.

Thus:

M. p., °C.	Grams dissolved per 100 c.c.
71	0.008
72	0.006
73	0.005

From the foregoing and other figures, the author has drawn up Table III giving the corrections for practical working and as a result of his experiments he has modified the process as given below.

TABLE III.

Weight of acids (corrected for 90% alcohol)	Correction per 100 c.c., 70% alcohol		
	M. p., 71°	M. p., 72°	M. p., 73°
Above 0.10 grm.	0.013 grm.	0.008 grm.	0.006 grm.
0.08–0.10 grm.	0.011 grm.	0.007 grm.	0.005 grm.
0.05–0.08 grm.	0.009 grm.	0.007 grm.	0.005 grm.
0.02–0.05 grm.	0.007 grm.	0.006 grm.	0.005 grm.
Less than 0.02 grm.	0.006 grm.	0.005 grm.	0.004 grm.
Factor for conversion of percentage of fatty acids to arachis oil.	17	20	22

Modified Renard's Process.—Weigh out 5 grm. of the oil into a flask, add 25 c.c. of alcoholic potassium hydroxide (80 grm. potassium hydroxide dissolved in 80 c.c. water and diluted to a litre with 90% (by vol.) alcohol), and saponify for about 5 minutes under a reflux condenser. To the hot soap solution add 7.5 c.c. of acetic acid (1 vol. of glacial acetic acid to 2 vol. of water) and 100 c.c. of 70% alcohol containing 1% (by vol.) of HCl, and cool to 12° to 14° for an hour. Filter and wash with 70% alcohol containing 1% of HCl at 17° to 19°, the precipitate being broken up occasionally by means of a platinum wire bent into a loop. The washing is continued until the filtrates give no turbidity with water, the washings being measured. Dissolve the precipitate, according to its bulk, in 25 to 70 c.c. of hot 90% alcohol, and cool to a fixed temperature between 15° and 20°. If crystals appear in any quantity, allow to stand at this temperature for 1 to 3 hours, filter, wash

with a measured volume of 90% alcohol (about half the volume used for crystallisation), and finally with 50 c.c. of 70% alcohol. Wash the crystals with warm ether into a weighed flask, distil off the ether, dry at 100°, and weigh. If the melting point is lower than 71°, recrystallise from 90% alcohol. Add the correction for the solubility in 90% alcohol as in Renard's process, from the table given by Archbutt (see Vol. II, p. 94), and also for the total volume of 70% alcohol used in the precipitation and washing (including the 100 c.c. added in the first instance) from Table III.

If there are no crystals from 90% alcohol, or only a very small amount, add a sufficient quantity of water to reduce the strength of the alcohol to 70% (31 c.c. of water to 100 c.c. of 90% alcohol). Crystallise at 17° to 19° for an hour, filter, wash with 70% alcohol and weigh as before, adding the correction for 70% alcohol from Table III. If the melting point is below 71° recrystallise from a small quantity of 90% alcohol, or again from 70% alcohol.

<div align="center">TABLE IV.</div>

Oil	Alcohol used for crystallisation, %	Weight of crystals	Correction for 90% alcohol	Correction for 70% alcohol	Total	%	M.p., °C.	% of arachis oil by factor
		Grm.	Grm.	Grm.	Grm.			
Arachis (A)....... {	90	0.160	0.040	0.027	0.227	4.54	73	100
	70	0.218	....	0.065	0.283	5.66	71	96
Arachis (B)....... {	90	0.163	0.045	0.032	0.240	4.80	72	96
	70	0.233	....	0.068	0.301	6.02	71	102
Arachis (C)........	90	0.152	0.054	0.034	0.240	4.80	72	96
Arachis (D)........	90	0.194	0.033	0.028	0.255	5.10	72	102
Arachis (A), 50%.... Olive "Nice," 50%....	90	0.056	0.032	0.022	0.110	2.20	73	48
	70	0.090	....	0.055	0.145	2.90	71	49
Arachis (A), 35%.. ⎫ Olive "Nice," 65%. ⎬	90	0.045	0.020	0.029	0.094	1.88	71	32
	90	0.029	0.040	0.020	0.089	1.78	72.5	37
⎭	70	0.059	....	0.040	0.099	1.98	71	34
Arachis (A), 20%.... Olive "Nice," 80%...	90	0.024	0.012	0.019	0.055	1.10	71	19
	70	0.030	....	0.024	0.054	1.08	71	18
Arachis (C), 20%.... Olive "Malaga," 80%	90	0.012	0.020	0.015	0.047	0.94	72	19
	70	0.021	....	0.027	0.048	0.96	71	16
Arachis (A), 10%.... Olive "Nice," 90%...	90	0.009[2]	0.008	0.008	0.025	0.50	73	11
	70	0.008	....	0.015[1]	0.023	0.46	70	8
Arachis (B), 10%.... Olive "Nice," 90%...	90	0	....	....	....	0	....	....
	70	0.012	....	0.018	0.030	0.60	71	10
Arachis (C), 10%.... Olive "Malaga," 90%	90	0	....	....	....	0	....	....
	70	0.011	....	0.016	0.027	0.54	71	9
Arachis (A), 5%.... ⎫ Olive "Nice," 95% ⎬	70	0.007	....	0.012[1]	0.019	0.38	....	6.5
Sesame........... {	90	0	....	....	....	0	....	....
	70	0.012	....	....	....	0.24	64	....
Cottonseed........ {	90	0	....	....	....	0	....	....
	70	0.006	....	....	....	0.12	50-55	....
Olive "saponified". {	90	0.014	....	....	....	0.28	64-67	....
	70	0.021	....	....	....	0.42	64-68	....

[1] In these cases the correction has been added for melting point 71°.
[2] This result was obtained by recrystallising the fatty acids obtained from 70% alcohol from 10 c.c. of 90% alcohol.

The results obtained by this method are given in Table IV.

The following oils gave no crystals: Olive oils, including "Nice superfine," "Nice seconds," "Malaga," and eight of unknown origin, almond, poppy and rape oils. "Saponified" olive oil on the other hand usually gives crystals (see olive oil, page 132).

The qualitative method of Bellier has been shown by Franz and Adler[1] to be capable of yielding approximately quantitative results by determining the temperature at which turbidity is first produced. For this purpose they give the following table of "temperatures of crystallisation."

Oil	Temperature of crystallisation	Oil	Temperature of crystallisation
	°C.		°C.
Olive oil.....................	11.8–14.3	Arachis oil, 50%.............	33.8
Arachis oil, 5%.............	15.9–17.0	Arachis oil, 60%.............	35.3
Arachis oil, 10%.............	19.8	Arachis oil, 70%.............	36.6
Arachis oil, 20%.............	25.7	Arachis oil, 80%.............	38.0
Arachis oil, 30%.............	29.2	Arachis oil, 90%.............	39.3
Arachis oil, 40%.............	31.5	Arachis oils.................	40.0–40.8

The present revisers have had the opportunity of noting these "temperatures of crystallisation" for a considerable number of mixtures and find them to give most useful indications, which are approximately correct in the majority of cases. In connection with this application of the test, H. Lüers[2] draws attention to a turbidity given by certain olive oils which were proved to be free from arachis oil, and he states that the addition of 3 drops of glacial acetic acid, in addition to the dilute acetic acid, prevents the formation of this turbidity.

ALMOND AND APRICOT-KERNEL OILS.

Ross and Race[3] have compared certain analytical figures for these two oils, and for the fatty acids obtained from them by fractional distillation; from their results they deduce that the composition of the two oils is so similar that they may, for practical purposes, be considered identical.

This statement is most unfortunate and misleading, for it is obvious that the purchaser of almond oil would be greatly defrauded if he were to be sold apricot-kernel oil, which is usually less than half the price. C. A. Hill (*ibid.*) records his disagreement with the statement that the oils may be considered as·identical. The same authors have shown that notwithstanding the similarity in general composition, apricot-kernel oil is distinguished by means of the Bieber reaction (Vol. II, p. 104) and that the chromogenic substance, which is not volatile, is not destroyed by subjecting the oil to steam distillation for some hours. Moreover, they found that even in the case of a sample a year old, through which air was blown while warm for 3

[1] Abs. *J. Soc. Chem. Ind.*, 1912, 30, 691.
[2] *Zeit. Unters. Nahr, Genussm.*, 1912, 24, 683.
[3] *Analyst*, 1911, 36, 263.

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days, the Bieber reaction was still so strong that as little as 5% could be detected when mixed with almond oil.

The following table gives the limits of the figures obtained from the analysis of 4 samples of almond and 3 samples of apricot-kernel oil bought commercially and for 1 sample sold as peach-kernel oil.

	Limits of four samples of almond oil	Limits of three samples of apricot-kernel oil	Peach kernel
Iodine value..................................	97 to 102	100 to 106	101.6
Saponification value........................	183.3 to 207.6	184 to 192.4	191.7
Sp. gr., $\frac{15°}{15°}$................................	0.9178 to 0.9199	0.9198 to 0.9200	0.9167
Ref. index at 40° (Zeiss).....................	57.5° to 58°	57° to 58.5°	55.5°
Bieber reaction..............................	nil.	strong	strong

FATTY ACIDS.

Saponification value.........................	200.4 to 207	197 to 202	201.6
Ref. index at 40° (Zeiss)....................	56° to 58°	57° to 59°	53°

The acidity of 23 samples of almond oil has been determined by J. C. Umney[1] who found it to range from 0.6 to 9.2% for acid calculated as oleic, and that an oil of high acidity was satisfactory in odour and lustre after keeping 6 months. Apricot oil, on the other hand, was not satisfactory in these respects after 12 months. 34 samples of peach and apricot-kernel oils were found to have acidities ranging from 0.6 to 5.97% (as oleic acid). Lewkowitsch[2] gives the following table of figures.

Description of oil	Sp. gr.	Saponification value	Iodine value.	Butyro-refracto-meter at 40° C. Degrees.	Free fatty acids[3]	Fatty acids.		Bieber's test
						Neutral-isation value	Saponification value	
Almond oils expressed from:								
1. Valencia sweets.....	0.91905	207.6	99.4	57.5	2.61	207.8	207.6	Colourless
2. Blanched valencia sweets.	0.9182	191.7	103.6	57.5	1.46	196.4	201.7	Colourless.
3. Sicily sweets........	0.9178	183.3	100.3	57.0	0.39	198.8	202.2	Colourless.
4. Mazagan bitters....	0.9180	188.6	102.5	56.5	1.56	196.8	203.1	Colourless.
5. Small Indian almonds.	0.91907	189.2	96.65	57.0	1.46	195.8	200.7	Colourless.
6. Mogador bitters....	0.9183	194.98	104.2	57.0	0.65	197.1	203.2	Colourless.
7. Peach-kernel oil....	0.9198	191.4	95.24	57.5	1.51	196.8	205.0	Colourless at first, then pink.
8. Apricot-kernel oil...	0.9200	192.4	107.4	58.0	1.16	198.0	202.0	Pink colouration.
9. Apricot-kernel oil from Mogador kernels.	0.9172	198.2	107.9	57.0	1.41	194.0	200.7	Slightly pink.
10. Californian apricot-kernel oil.	0.92026	190.3	108.7	58.0	0.61	197.8	202.8	Very slightly pink.

[1] *Perf. and Essent. Oil Record*, 1914, 5, 101.
[2] *Analyst*, 1904, 29, 106.
[3] Calculated by present revisers from acid values.

OLIVE OIL.

Rape oil in olive oil is best detected by **Tortelli and Fortini's method.**

In carrying out this test the following details must be adhered to *exactly.* 20 grm. of the oil are saponified with 6 grm. of potassium hydroxide dissolved in 50 c.c. of 90% alcohol by heating under a reflux condenser. The liquid is neutralised to phenolphthalein with 10% acetic acid and the solution then slowly poured into a boiling mixture of 200 c.c. of 10% lead acetate and 100 c.c. of water, shaking vigorously during the addition. The mixture is cooled under the tap, maintaining a rotary motion until the soaps begin to stick to the sides (if they do not stick at first, they will do so during the first washing). The water is poured off and the soaps washed 3 times with 200 c.c. of warm (60 to 70°) water, the water being then drained off and, as far as possible, removed with filter paper. To the dried soaps 80 c.c. of methylated ether are added and the whole is well shaken for several minutes till the mass is broken up, when it is heated under a reflux condenser for 30 minutes, shaking at intervals. The flask is then closed and placed in water *at exactly* 15° C. *for* 1 *hour*, after which the contents of the flask are poured on to a filter, the funnel being placed in the mouth of a separator and the filter closely covered till all the ether possible has filtered out. The filter and contents are dropped back into the flask and the ether treatment (boiling and cooling) is repeated in exactly the same way, using 40 c.c. of ether, and the mass filtered as before, again tightly covering the filter and allowing to drain as completely as possible. The flask is washed out with a further 40 c.c. of ether on to the filter, the contents of which are well stirred up with the ether, which is then allowed to drain off. The combined ethereal solutions of the lead salts so obtained are decomposed in the separator by shaking twice with 150 c.c. of 10% hydrochloric acid, after which the ether is washed with two quantities of 100 c.c. of water, the ethereal solution being then run out into a dry flask and allowed to evaporate spontaneously or by the use of gentle warmth in a current of hydrogen. The liquid fatty acids so obtained are dissolved in 40 c.c. of strong alcohol (97 %) and a saturated solution of sodium carbonate added until the liquid is saturated (sodium carbonate separates). The alcohol is distilled off and the residue dried, first in the water oven, distributing it as much as possible over the sides of the flask, and finally in a vacuum desiccator for at least 48 hours. The dry sodium soaps are then boiled with 50 c.c. of *absolute* alcohol and filtered in a hot funnel, the insoluble residue being boiled with a further quantity of alcohol and the treatment repeated till nearly the whole has been dissolved. The mixed alcoholic filtrates are freed from alcohol by distillation and the sodium soaps dried as completely as possible in a vacuum desiccator over sulphuric acid.

According to Tortelli and Fortini the test is concluded as follows:

0.5 grm. of the dry soaps are placed in a large test-tube and dissolved by

heating in 20 c.c. of absolute alcohol. A thermometer is then placed in the mixture, which is allowed to cool and the turbidity temperature noted.

The following table gives some results obtained by them:

Oil	Turbidity temperature, °C.
Olive...	20–24°
Rape..	45–50°
1 pt. olive 1 pt. rape	35–40°
8 pt. olive 2 pt. rape	30–35°
9 pt. olive 1 pt. rape	30–34°
Cotton..	14–16°
Sesame...	18–20°
Arachis...	18–22°

The writers find it more satisfactory to dissolve 0.75 grm. of the soaps in strong alcohol (97 to 98%) and to leave the solution to stand at a temperature of 20° C. Under these circumstances rape oil commences to precipitate in a granular form in 15 to 30 minutes, and 5 to 10% of rape oil in admixture with other fats produces a spongy gelatinous precipiate within 2 hours, while in the absence of rape oil no precipitate usually forms under 15 to 18 hours. As the results are dependent on the degree of dryness of the soaps and the strength of the alcohol employed it is more satisfactory to carry through the test with some oil, such as cottonseed oil, as a control. The test under these conditions is quite reliable.

To detect small quantities of cottonseed oil, the present revisers recommend that the Halphen test be carried out in sealed tubes as suggested by Steinmann, while for still smaller quantities (down to 1%) R. Marcille[1] proposes that the sealed tubes be heated in an oil bath at a temperature of 120° when 5 to 10% of cottonseed oil give a bright red colour within 12 minutes, and 1% a distinct red after 1 hour.[2] Attention is drawn to the fact that certain olive oils give a red colour when heated to 130° and for this reason care must be taken not to exceed 120° which temperature is stated not to produce any red colour with pure olive oil after 6 hours heating, though a slight yellow tint is usually observed.

"Saponified" olive oils are liable to be taken as adulterated with foreign oils on account of the different analytical figures which they give, and more particularly on account of the large precipitate which they yield when subjected to Bellier's test (see arachis oil). Archbutt[3] has investigated this point and has shown that one particular sample of oil of this type which contained 3.2% of unsaponifiable matter and gave a precipitate by Bellier's test, yielded no trace of arachidic acid by Renard's process and another sample which gave an unusually marked indication by Bellier's test, was found by Renard's method to contain less than 5% of arachis oil. Archbutt points out the necessity of a careful interpretation of this test and due confirmation by Renard's method before the presence of arachis oil be certified.

[1] Ann. Falsific., 1910, 3, 235.
[2] Other proposed improvements in this test will be found under cotton oil, page 135.
[3] J. Soc. Chem. Ind., 1911, 30, 5.

Attention is drawn to the turbidity of these oils, which is usually due to the presence of moisture, and to the increased viscosity, together with various other analytical differences exemplified in the following table of analyses of nine samples examined by him.

Sp. gr. at 60° F	0.9167	0.9165	0.9175	0.9186	0.9169	0.9175	0.9169	0.9161	0.9179
Efflux time of 50 c.c. from Redwood's viscometer at 60° F. (seconds)	516.0	450.0	437.0	478.0	480.0	561.0	524.0	428.0	465.0
Saponification value	186.1	186.9	189.3	186.9	186.0	185.5	185.9	185.4	185.8
Free (oleic) acid	2.9	3.3	1.0	1.1	0.9	2.3	1.8	0.4	1.6
Iodine value	86.4	85.1	85.8	84.4	85.5	85.6	85.0	86.5	86.2
Unsaponifiable matter	2.49	2.34	2.08	2.70	3.30	3.32	3.23	2.98	2.67
Arachidic acid, by Renard's process	......	......	......	......	......	......	......	......	......

"Extracted" olive oil, obtained by means of carbon disulphide, is liable to contain traces both of this solvent and of free sulphur. The latter may be detected by warming a silver coin or strip of copper in the oil. Carbon disulphide may be detected by the method suggested by E. Millau[1] who distils 50 grm. of the oil with 10 c.c. of pure amyl alcohol, collecting the first 5 c.c. of the distillate and to 4 c.c. of the distillate adds 1 c.c. of kapok or cottonseed oil together with a few mg. of sulphur, heating the mixture in a sealed tube for 1 hour.

The present revisers found that as little as 0.05% of carbon disulphide can be easily detected in this way. Olive oils which have been extracted by carbon disulphide have been examined by F. Canzoneri and G. Bianchini[2] who found them to differ from the ordinary expressed oil in the following respects:

(1) High specific gravity.

(2) Lower solidification of the fatty acids (e.g., 17.5° to 19.7°).

(3) Lower iodine value (77.5 to 80.2).

(4) High acetyl value.

(5) Lower refractive index (59° to 61° Zeiss) except in the case of oils bleached by oxidation, in which the reading exceeds 63°.

(6) Saponification value lower than normal.

O. Klein[3] has examined 30 samples of oil prepared from known varieties of olives and 30 commercial samples from which he deduces the following limiting values for Portuguese oils:

	Sp. gr.	Refractive index (25° C).	Iodine value	Saponification value
Maximum	0.918	1.4682	85.0	195.0
Minimum	0.915	1.4660	75.0	185.0
Average	0.9168	1.4670	80.5	190.0

[1] Ann. chim. anal., 1912, 17, 1.
[2] Annali. Chim. Applic., 1914, 2, 1.
[3] Chem. Zentr., 1912, 1, 1664.

TEA SEED OIL.

The following figures have been obtained by the present revisers for a sample of commercial oil obtained from Chinese tea seed.

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Sp. gr. at 15.5°..............................................  0.9163
Iodine value..................................................  84.35
Refractive index at 40° C. (Zeiss) ...........................  53.8
Saponification value..........................................  190.5
Free fatty acids (as oleic)...................................  1.84
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Menon[1] has examined seeds from Upper Assam which on extraction with petroleum ether yielded 16.1% of an oil having the following characteristics:

```
Oil:
    Sp. gr., 15°/15°..........................................  0.9028
    Saponification value......................................  189.9
    Reichert-Meissl value.....................................  0.56
    Titration number of insoluble volatile acids..............  0.56
    Iodine value..............................................  92.7

Fatty acids:
    Insoluble fatty acids + unsaponifiable....................  2.6
    Insoluble fatty acids.....................................  93.04
    Melting point.............................................  38.9
    Neutralisation value......................................  199.9
    Mean molecular weight.....................................  280.5
    Iodine value..............................................  94.13
```

The fatty acids consisted of about 25% of solid acids melting at 57.8° and having a neutralisation value of 209.8, mean molecular weight 267.3, iodine value 13.84, and 74.75% of liquid acids of the neutralisation value 191.1, mean molecular weight 293.5, and iodine value 117.8.

MUSTARD OIL.

Black Mustard Oil.—The black seeds contain about 30% of oil, which is usually obtained as a by-product from the manufacture of mustard.

The Indian oil is often adulterated with sesame and similar oils.

White Mustard Oil.—The white seeds contain about 25% of oil. The following figures have been recorded by Grimme for the oil obtained from four different kinds of seeds.[2]

	Sinapis arvensis L.	Sinapis chinensis L.	Sinapis dissecta L.	Eruca sativa Lmk.
Oil:				
Sp. gr. at 15° C.................	0.9228	0.9230	0.9221	0.9198
Solidifying point................	−13° to −15°	−14°	−13° to −14°	−8° to −10°
Saponification value.............	179.4	177.3	178.2	174.4
Iodine value....................	102.6	103.3	105.6	101.8
Refractive index at 20° C........	1.4738	1.4736	1.4725	1.4723
Fatty acids:				
Fatty acids, per cent............	94.21	94.28	94.34	94.24
Unsaponifiable matter, per cent...	1.12	0.96	0.96	1.07
Solidifying point................	4–5°	14–15°	5–8°	8–10°
Melting point...................	6–8°	17–18°	9–10°	12–13°
Neutralisation value............	179.8	182.0	181.7	180.1
Mean molecular weight...........	312.4	308.6	309.1	311.8
Iodine value....................	106.6	106.7	109.0	103.6
Refractive index at 20° C........	1.4625	1.4648	1.4645	1.4643

[1] Year Book of Indian Guild of Science and Technology, 1912, 144.
[2] Lewkowitsch, *Oils, Fats and Waxes*, 5th Ed., 2, 271.

The percentage of ethereal mustard oil obtained from the seeds of the above species are given in the following table:

Name	Ethereal mustard oil in seed, %	Ethereal mustard oil in extracted seed meal, %
Sinapis arvensis	0.959	1.308
Sinapis chinensis	1.407	2.022
Sinapis dissecta	0.833	1.150
Bruca sativa	1.075	1.586

A method for the estimation of essential oil of mustard is given in Abs. *J. Chem. Soc.*, 1912, ii, 308.

COTTONSEED OIL.

Various improvements have been suggested in order to render the Halphen test still more delicate (some of these are described under olive oil). One of the most important and one which the present revisers have found to be extremely delicate, is the substitution of pyridine for amyl alcohol, as suggested by E. Gastaldi,[1] who carries out the test by mixing in a strong test-tube, 5 c.c. of the oil or fat to be tested, 1 drop of pyridine and 4 c.c. of carbon disulphide containing 1% of sulphur, corking the tube and heating in the water-bath for half an hour. As little as 0.25% of cottonseed oil will be found to produce a distinct red tint if compared with a control tube.

Utz[2] proposes to substitute pentachlorethane (b. p. 159°) for carbon disulphide so as to obtain a higher temperature, and states that he has obtained a reaction with 1% of cottonseed oil and that the colour is produced without the presence of amyl alcohol, if the temperature is sufficiently raised. Gastaldi and others, however (*vide* olive oil), have shown that if the temperature is raised appreciably above 120° a red colour is often produced when no cottonseed oil is present. The statement of Utz must therefore be accepted with due reserve.

KAPOK OIL.

Known also under the name of Bastard cotton oil, is chiefly obtained from the seeds of *Eriodendron anfractuosum* which yields a fruit similar to that of the cotton plant, the chief distinction being that the seeds themselves are quite free from the hairs so characteristic of cotton seeds, and are small, round and black in colour—the hard shell constituting about 40% of the whole.[3] The tree abounds in Java, the West Indies, Africa, etc., where it is often termed the "silk cotton tree," the same name being applied to the East Indian tree, *Bombax malabaricum*, which is very similar and from which kapok oil is also obtained, there being no commercial distinction

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[1] Abs. *J. Soc. Chem. Ind.*, 1912, 31, 934.
[2] *Chem. Rev. Fett. Ind.*, 1913, 20, 291.
[3] Lewkowitsch.

drawn between the oils from these two sources. Sprinkmeyer and Died-richs[1] have examined the oils obtained from the various species in order to differentiate between them if possible, and some of the figures which they have obtained are given in the following table.

Source	Java, E. Africa Ceylon, etc.[2]	Bombax malabaricum[3]	Mexican Bombax[3] (variety)	Commercial oil[3]
Sp. gr. at 15/15°..................	0.9235– 0.9326	0.9600	....	0.9217
Ref. index at 40° (Zeiss)..........	51.7 – 59.7	57.0	57.4	56.2
Iodine value.....................	85.2 – 93.5	73.6	95.7	97.54
Acid value......................	18.5 –21.02	3.0	12.62	15.0
Saponification value..............	189.2 –194.5	194.3	192.8	192.5

Kapok oil is very like cottonseed oil in most respects and even gives the Halphen reaction to a slightly greater extent than the latter. Small quantities of kapok oil may, however, be detected in cottonseed oil by means of a modification of Becchi's test devised by Millau when applied in the form recommended by Durand and Band. The test is carried out as follows:

15 c.c. of the oil are saponified with sodium hydroxide and alcohol in the usual manner, 200 c.c. of boiling water are added and the whole boiled till the alcohol is evaporated. The fatty acids are then thrown out by the addition of $N/10$ sulphuric acid in slight excess. The fatty acids are skimmed off, and shaken twice with 15 c.c. of *cold* distilled water, the water being then drained off and the fatty acids dried rapidly in an oven at 105°. 5 c.c. of these fatty acids are shaken with 5 c.c. of a 1% solution of silver nitrate in absolute alcohol.

Under these circumstances cottonseed oil only produces a *barely perceptible brown colour*, while kapok oil rapidly develops a *deep coffee colouration*. By means of this test it is possible to recognise 1% of kapok oil in other liquid oils.

The imports of kapok seed to Holland and America are steadily increasing. The oil is used for the same purposes as cottonseed oil, with which it is often mixed, and increasing quantities are refined for edible purposes, more particularly in Holland.

The content of the oil in the whole seed ranges from 22% to, in some cases, 30%, while the kernels themselves usually contain about 40%. The seeds of *B. malabaricum* are generally rather higher in oil content.

SESAME OIL.

Attention has been drawn by Zimmermann[4] to the failure of highly refined sesame oil to give many of the usual colour reactions, and he states that Solsein's stannous chloride test is the least affected. In the experience of the present revisers it is possible to obtain a strong Baudouin reaction

[1] *Zeit. Unters. Nahr. Genussm.*, 1913, 26, 86, and 450.
[2] Sprinkmeyer and Diedrichs (*Z. Unters. Nahr. Genussm.*, 1913, 26, 86 and 450).
[3] *Fatty Foods*, p. 222.
[4] *Mitt. d. k. k. Techn. Vesuchsamtes.*, 1912, 1, 71.

from the refined oil, though some processes of refining do, as stated above, considerably diminish the sensitiveness of this test. At one time the German regulations (see Vol. II, p. 141) required 10% of sesame oil to be added to all butter substitutes to facilitate their detection, but owing to the aforesaid reduction in the sensitiveness of the Baudouin reaction, it was necessary subsequently to modify these regulations, and to require such an amount of sesame oil to be added as would suffice to give a distinct red colour under specified standard conditions (Vol. II, p. 316) without laying down any limits as to the quantity of sesame oil which might be required to fulfil these conditions.

SOJA-BEAN OIL.

The very large quantities of this oil which have come on the market in this country and more particularly in the U. S. A., render it of great commercial importance.

Many investigations have been carried out with the object of utilizing the oil for various purposes other than that of soap making and the recorded statements of different observers are most contradictory. This divergence of opinion may be explained by the great variety of different species of soja beans and it is hardly to be expected that they should all yield an oil having identically the same properties.

Maximilian Toch[1] has examined 33 different varieties of soja beans and he points out that in the records of the Department of Agriculture at Washington no less than 280 varieties of soja beans have been recorded. This investigator explains the controversial statements of other workers on the grounds that the oil from certain varieties of beans is suitable for use in paints (i.e., as a substitute for linseed oil) and goes on to draw a favourable comparison between those types and linseed oil, pointing that the type of oil adapted for use in paint possesses two characteristics, (a) that when heated to 500° F. for a few minutes, it will become bleached and remain bleached, and in this respect resembles linseed oil to a certain extent; (b) that when heated to 500° F. and blown with dry air for 5 to 7 hours, it thickens in a similar manner to linseed oil and attains a sp. gr. of 0.960 or over. The following figures were obtained by this author for a standard sample of cold-pressed Manchurian bean oil, which was heated to 500° F. and after cooling to 300° F. blown vigorously for 7 hours.

	Sp. gr., 60° F.	Acid value	Iodine value
Original oil..................	0.929	2.6	133.6
Blown oil..................	0.963	1.9	105.6

Blown soja oil is used in linoleum manufacture. The figures given in the following table were obtained in Messrs. Toch Bros.' research laboratory.

[1] *J. Soc. Chem. Ind.*, 1912, 31, 572.

Name	Colour of seed	Colour of oil	Sp. gr., 15° C.	Acid value	Iodine value
Meyer..................	Brown	....	0.9264	0.44	127.0
Peking................	Black		0.9279	0.14	135.4
Haberlandt............	Straw-yellow	Extremely pale.	0.9244	0.00	129.8
Farnham..............	Straw-yellow		0.9234	0.65	131.8
Taha..................	Black Olive	Pale amber some-what deeper than than above.	0.9248	0.16	127.0
Mammoth.............	Saddle Straw-yellow		0.9222	0.47	118.2
Edward...............	Straw-yellow	Med. amber.	0.9257	1.14	124.6
Shanghai..............	Black	Same depth as pre-vious olive tone.	0.9241	0.63	127.8
Refined linseed.........	....	....	0.933	1.0	180.1

Soja oil is used to a certain extent as an edible oil, but has not fulfilled anticipations, still while there is no difficulty in preparing a tasteless and odourless oil, it has been the experience of the present revisers that this does not keep very well, and has a tendency to develop an unpleasant "oxidised" taste.

CANDLE NUT OIL.

In addition to the seeds obtained from the South Sea Islands large quantities are exported from Hongkong and Fiji, as well as Australia and New Zealand.

The oil is known as "Kekune" or "Country Walnut oil," etc.

The seeds of *Aleurites triloba* are said to produce an edible oil, but that obtained from *Aleurites moluccana* has, as has been pointed out by Lewkowitsch, purging properties. The seeds from both varieties are sold indiscriminately under the names of "Candle nuts," "Baio nuts," "Lumbang nuts," etc.

The present revisers (*Fatty Foods*, page 251) have extracted samples of the oil from authentic specimens of the seed of both varieties and have obtained the following figures:

	Aleurites moluccana	Aleurites triloba[1]
Saponification value...	190.3	202.5
Iodine value..	164.0	143.8
Ref. index at 40° C. (Zeiss scale).....................................	65.7	61.8
Free fatty acids (as oleic)..	20.1%	1.0%

If these figures are compared and contrasted with those of other observers (*vide* Vol. II, 149) it would appear that the oil from *Aleurities triloba* has a higher saponification value and a lower iodine value than that of *Aleurities moluccana*.

A sharp distinction must be drawn between Candle nut oil and Tung oils,

[1] The oil remained liquid below zero without depositing "stearine."

though the Chinese variety of tung oil (*vide* page 140) is obtained from closely allied species of Aleurities (var. Fordii and Montana). Tung oils are decidedly poisonous.

HEMP SEED OIL.

Hemp seeds contain about 33–35% of oil.

The following figures were obtained by the revisers from one genuine sample:

Sp. gr. 15 /15°...	0.9283
Saponification value......................................	191.0
Iodine value..	161.7
Ref. index at 40° (Zeiss).................................	73.5
Free fatty acids, per cent................................	2.3

PINE NUT OIL.

The data in the following table have been collected by Lewkowitsch.[1]

Oil from	Yield oil, %	Sp. gr. at 15°	Solidifying point	Saponi-fication value	Iodine value (Wijs)	Ref. index	Observer
Pinus sylvestris, L........	32.1	0.9326	−28 to −29	189.8	147.1	1.4704 at 35°	Grimme.
Pinus montana, Mill......	29.6	0.9318	−25 to −26	189.6	145.7	1.4698 at 35°	Grimme.
Pinus cembra, L..........	......	0.930	−20	191.8	159.2	....	v. Schmoelling.
Pinus cembra, L..........	35.7	0.9316	−20 to −21	188.0	156.3	1.4710 at 40°	Grimme.
Pinus picea, L...........	32.8	0.9268	−25 to −26	190.5	120.9	1.4879 at 35°	Grimme.
Pinus abies, L...........	31.6	0.9312	−26	192.0	120.5	1.4742 at 35°	Grimme.
Pinus Gerardiana, Wall...	30.7	0.9307	−17	191.3	120.9	1.4685 at 40°	Grimme.
Pinus pinea, L...........	21.8	0.9326	−22	192.6	118.3	1.4679 at 35°	Grimme.
Cupressus sempervirens v. horizontalis, Mill. }	10.8	0.9320	−4	188.6	135.1	1.4857 at 35°	Grimme.
Thuja occidentalis, L.....	15.0	0.9298	−8	186.7	154.8	1.4795 at 35°	Grimme.
Pinus monophylla...... *Pinus Fremontiana*..... }	......	0.933	....	192.8	101.3	1.4769(?)	Blasdale.
Pinus monophylla...... *Pinus Fremontiana*..... }	......	......	....	189.0	108.0	1.4543 at 40°	Adams and Holmes.

POPPY SEED OIL.

The oils obtained from different varieties of poppy seed are divided commercially under two headings:

(1) "*Huile d'œillette*," obtained from the grey or blue European seed.

(2) "*Huile de pavot*," from the brown or mottled seeds of foreign origin.

"Huile d'œillette" is the better oil and commands a higher price.

The following simple test serves to distinguish the two types of oil.

The oil is violently shaken in a bottle when it will be found that (1) gives a fine emulsion of air bubbles, rendering the oil turbid, (2) behaves quite differently and does not give a fine emulsion nor is the froth so persistent.

"Huile d'œillette" possesses a much more golden-yellow colour than "huile de pavot," and so much so, that it is often necessary to colour the latter in order to render it saleable.[2]

SAFFLOWER OIL.

The oil is obtained from 2 distinct varieties, *Carthamus tinctorius* and *Carthamus oxyacantha* the latter being "wild" safflower. The seeds are

[1] *Oils, Fats and Waxes*, 5th Ed., Vol. 2, p. 141.
[2] L. Vuaflart, *Ann. Falsif.*, 1909, 2, 276.

commonly known as "Kurdee" or "Kardai" seeds, and the quantities expressed are largely on the increase. The oil-cake produced, from the decorticated seed, contains nearly 50% of protein. The figures in the following table have been obtained by Leather.[1]

District	No. of samples	Oil, %	Wt. of 100 seeds in grm.
Central Provinces...............	6	23.54–31.82	3.405–6.774
Bombay Presidency..............	9	28.79–32.23	4.210–5.516
Madras Presidency..............	8	23.88–33.55	2.973–4.622
United Presidency..............	6	27.94–29.78	3.348–4.936
Bengal........................	1	22.47	3.209

The oil gives a marked hexabromide reaction and is a good drying oil being used by the natives in parts of India for linoleum manufacture.

SUNFLOWER OIL.

This oil is usually obtained from the fruits of the *common* sunflower, and seldom from the seeds alone as is generally supposed. It should be noted that the portion usually termed "the seed" is really the whole fruit. The plant is indigenous to Mexico, but is extensively cultivated in Russia, China and Hungary and is so abundant in South Africa that it is used to mark out the boundaries of fields. Notwithstanding the fact that it is a very easy plant to grow and produces an enormous yield of fruits, the attempts to introduce it into India and the United States have not proved very satisfactory and the crop in Great Britain is too small to be of any commercial importance, though the climatic conditions lend themselves to its production. It appears that the value of the crop is not realised by the conservative British farmer.

Sunflower oil serves well for edible purposes though it is a little difficult to refine owing to its tendency to form emulsions.

The fruits vary in colour from white to a dark brownish black, and contain about 22–25% of oil (45–50% calculated on the true seed). The oil from the white fruits has been found by the present revisers to have a much lower iodine value (106) and a lower refractive index than that yielded by the black seeds.

TUNG OILS.

Chinese Wood Oil.—The enormous increase in the use of this oil of late years has caused it to be the subject of many investigations, one of the chief objects in view being to arrive at some satisfactory method of assaying its purity, and to set up a standard specification for purpose of sale, to which all pure samples should conform. As might be expected, a number of quite useless tests have been put forward.

[1] *Mem. Depart. Agric.*, India, March, 1907.

A general survey of the more important methods has been made by Chapman[1] whose paper should be consulted. He points out that the analytical determinations of the greatest importance are the sp. gr., the iodine value, the refractive index, the viscosity and the polymerisation test.

The Wijs method of determining the iodine value is recommended, if carried out in the following manner:

About 0.1 grm. of the oil is dissolved in 20 c.c. of purified carbon tetrachloride, 30 c.c. of the Wijs solution added and the absorption allowed to proceed for 3 hours in the dark.

In connection with the viscosity (time of efflux) the same author draws attention to the fact that the viscosity of tung oil is greater than that of any other fatty oil likely to be used as an adulterant, but the warning is added that the viscosity may be considerably increased by heating the oil to a temperature short of that required for solidification.

Polymerisation Tests.—A great number of the tests put forward are based on the property which this oil possesses of setting to a firm mass when heated. It has been suggested to solidify the oil by heating it under standard conditions and to grind and extract the mass with ether, but the present revisers have found this to be most misleading and unsatisfactory except in the case of gross adulteration which could be more easily detected by other methods.

In a circular issued by the New York Produce Exchange a method devised by C. V. Bacon is tentatively put forward.

"Into a test-tube ¾ in. diameter and 4 in. in length there are transferred about 10 c.c. of pure China wood oil; into another test-tube there is transferred a similar volume of pure China wood oil adulterated to the extent of 10%. A sample of the oil to be tested is treated in a like manner, and these are placed in a proper support and immersed in an oil bath which has a temperature of about 288° C.; so that when the tubes are in it a temperature of 280° or 285° C. (maximum) can be maintained. The oil bath containing the tubes is maintained at this temperature for exactly 9 minutes, the tubes are then withdrawn and the test sample is compared with the pure oil, and with the same oil adulterated with 5 and 10% of foreign oil. After the tubes are withdrawn from the oil bath, each tube should be stabbed from top to bottom with a small bright spatula. Pure oil will give a hard, clean cut, and when the knife is withdrawn the incision will look like a straight line, but an oil having an adulteration as low as 5% will invariably be softer, and the incision will have a peculiar feathered effect; whilst an adulteration of 10% will be soft and "pushy," an adulteration exceeding 12% in many instances will remain entirely liquid."

A further test which is used by the New York importers and varnish makers is described as follows:

"Hankow and Shanghai wood oil, 100 grm., should be heated in an

1 *Analyst*, 1912, 37, 543.

open basin (6 in. in diameter) as soon as possible to a temperature between 540° and 560° F. and if it solidifies in about 6 to 6½ minutes, cuts dry, and is firm in body, without discolouration and without being sticky, it should be passed as a good delivery. For Canton and Hongkong wood oil deliveries, the time should be from 4½ to 5½ minutes in an open basin as above. Should a longer time be taken by what is presumably pure wood oil, other tests confirming purity shall be positive."

Chapman (*ibid.*) criticises these tests and as a result of considerable experience states that he attaches more importance to the hardness of the jelly obtained under standard conditions than the time required for bringing about polymerisation. He has devised the following method of carrying out the test, which he finds to be capable of yielding definite and concordant results:

In the following table he gives the results of the examination of 17 samples of Chinese wood oil from Hankow:

Sample	Iodine value	Sp. gr. 15°/15°	Saponification value	Ref. index at 20°	Time of efflux at 15.5°, seconds	Polymerisation 1 hour at 250°
No. 1.............	169.9	0.9419	196.6	1.5207	2,178	Very hard.
No. 2.............	168.4	0.9406	193.8	1.5181	1,636	Hard.
No. 3.............	166.5	0.9426	194.3	1.5190	1,946	Fairly hard.
No. 4.............	166.4	0.9417	193.0	1.5170	1,880	Fairly hard.
No. 5.............	168.8	0.9430	195.6	1.5195	2,017	Very hard.
No. 6.............	170.0	0.9440	194.5	1.5180	1,849	Hard.
No. 7.............	168.6	0.9416	193.0	1.5150	....	Fairly hard.
No. 8.............	171.0	0.9414	192.0	1.5170	....	Hard.
No. 9.............	169.7	0.9437	194.1	1.5176	1,997	Hard.
No. 10.............	173.0	0.9420	192.5	1.5165	1,722	Hard.
No. 11.............	176.2	0.9417	192.0	1.5168	1,605	Hard.
No. 12.............	172.6	0.9429	196.0	1.5180	1,740	Hard.
No 13	174.2	0.9427	194.6	1 5182	1,690	Hard.
No. 14	173.7	0.9430	195.0	1.5194	1,820	Hard.
No. 15.............	172.8	0.9440	194.6	1.5193	2,047	Hard.
No. 16.............	169.5	0.9420	195.2	1.5160	1,804	Hard.
No. 17.............	169.6	0.9433	195.2	1.5187	1,820	Very hard.
Average.............	170.6	0.9425	194.2	1.5179	1,850	

The following extract has been taken from his paper:

"About 5 c.c. of the oil to be examined are introduced into each of 2 test-tubes 6 in. long by ⅝ in. diameter. These are then immersed in a bath containing melted paraffin wax at a temperature of approximately 100°. The temperature of the bath is then raised to 250°, taking about 15 minutes for the operation. As soon as that temperature is reached the time is noted, and the source of heat adjusted so that the temperature of the bath is maintained constant at 250°. At the end of half an hour one of the tubes is withdrawn, allowed to cool, and, when cold, is broken, and the jelly examined. The other tube is kept in the bath at 250° for a further period of half an hour, at the end of which time it also is withdrawn and allowed to cool; it is then broken, and the hardness of the jelly observed. Chinese wood oil of good quality should give at the end of half an hour a fairly firm jelly, which, at the end of 1 hour, should become quite hard. It is advisable in all cases to carry out comparison tests alongside of the oil under examination, using for the purpose a sample of oil known to be of good quality.

"I have not found it possible to express the hardness of the solidified cylinders by means of numbers, but with a little experience it is very easy to distinguish between a sample of

genuine oil and the same oil containing a small percentage of some fatty oil, such as soja bean or sesame. In referring to the polymerisation experiments, I have used the words "very hard," "hard," and "fairly hard," to denote the consistency of the polymerised oil, since such expressions are quite sufficient for the purpose. In addition to the degree of hardness of the solid cylinders of oil, some attention should be given to their physical characters. When cut with a knife or broken across, the cut or fractured surface should be smooth and free from stickiness, and small portions when rubbed in the hand should break down completely into a soft crumbly mass, which should not adhere to the fingers."

Hexabromide Test.—This test becomes of considerable importance for the detection of other oils which yield insoluble hexabromides, such as fish or marine animal oils as well as linseed, rubberseed oil, etc., for it has been shown by Hehner and Mitchell and independently by Jenkins and Chapman (the latter having worked on the 17 samples above referred to as well as on 4 samples of Japanese wood oil) that no insoluble hexabromides are obtained by the methods proposed by Hehner and Mitchell.[1] The present revisers have applied this test, as modified by Halphen,[2] to a large number of samples without obtaining a precipitate in any one case.

Candle nut oil (page 138) obtained from another species of *Aleurites* as well as perilla and hemp seed oils yield notable quantities of insoluble hexabromides.

Ware and Schumann,[3] give the following methods to detect adulterating oils, which is based on the insolubility of the potassium soaps of chinese wood oil in absolute alcohol. 3 grm. are saponified for 30 minutes under a reflux condenser with 100 c.c. of $N/4$ absolute alcoholic potassium hydroxide and the soap solution is cooled for 10 minutes at 0° and filtered through a Gooch crucible surrounded by ice. The precipitate is washed with ice-cold absolute alcohol previously saturated with the potassium soap of elæo-margaric acid and the residue dried *in vacuo* at 75° to 80°, in a current of hydrogen or carbon dioxide and weighed. The weight of the dry insoluble soap may be taken as measuring the wood oil in the sample.

Experiments on test samples containing from 5 to 40% of linseed and soja bean oils gave results within 1 to 2% of the theoretical.

	1	2	3
Sp. gr. 15.5°/15.5°............	0.9406	0.9396	0.9276 to 0.9416
Ref. index at 25°............	1.5143	1.5186	1.4790 to 1.5200
Moisture and volatile matter...	0.012 %	0.02 %	....
Ash......................	0.0068%	0.0026%	....
Acid value...............	3.45	0.90	0.2 to 0.8
Saponification value..........	192.27	193.02	188.2 to 192.4
Unsaponifiable matter........	0.73 %	0.47 %	....
Iodine value (Hubl, 18 hours)..	169.3	169.6	151.6 to 171.7
Iodine-jelly test[4].............	3 min. 37 sec.	4 min. 43 sec.	3 min. to 8 min.
Heating test[5]................	9 min. 54 sec.	9 min. 23 sec.	10 min. to 11.5 min.

[1] *Analyst*, 1898, 23, 310.
[2] *Fatty Foods*, page 42.
[3] *Proc. Amer. Soc. Test. Mat.*, 1914.
[4] *Iodine-jelly Test.*—This test is carried out by mixing 1 grm. of the oil with 5 c.c. of chloroform at 25°, adding 5 c.c. of a saturated solution of iodine in the same solvent and stirring the mixture until a jelly is formed. The time is noted from the addition of the iodine to the formation of a jelly.
[5] *Heating Test.*—5 c.c. of the oil are placed in a test-tube containing a glass rod and heated in an oil-bath at 282°, the rod is raised after 9 minutes and afterwards at intervals of 50 seconds, the time being noted when a jelly is formed.

In a report of the same Society[1] the figures in the foregoing table are given for two samples of Chinese wood oil, No. 1, being commercially obtained from the exporter and No. 2 expressed in the laboratory from Chinese wood oil nuts.

In column 3 is a summary of the average results of 11 investigators upon 3 samples of tung oil pressed from American grown nuts.

Japanese Wood Oil.

With regard to the source of Japanese wood oil considerable divergence of opinion exists as to the exact botanical species from which it is obtained. While Lewkowitsch,[2] states that the oil is obtained from the fruits of *Elæo-cocca vernicia*, quoting Kametaka[3] and distinctly states that this tree differs from *Paulownia imperialis*, Chapman (supra) examined oil extracted by himself from the fruits of the latter plant and obtained values closely agreeing with samples of Japanese wood oil obtained from Japan. Later, however, Wilson[4] states that the seeds actually examined by Chapman were those of *Aleurites cordata* and not those of *Paulownia imperialis*. In view however of the fact that Chapman himself examined oil extracted in his own laboratory from the seeds of *A. cordata*, it seems difficult to make these statements harmonise.

In a private communication to the present revisers, Chapman states that the oil examined by him was prepared from seeds forwarded to him from an authentic source in Japan and from a district in which the oil was being commercially manufactured and he was informed that the seeds in question were obtained from *Paulownia imperialis* and he further states that the seeds in question were quite different from those of *Aleurites cordata* in his possession, one specimen of which had been received from the Imperial Institute. In view of these facts, the present revisers are of the opinion that Chapman's statement as to the botanical source of these seeds must be taken as correct, though whether *Paulownia imperialis* is to be considered a botanical synonym for *Aleurites cordata* must be left an open question.

Whatever may be the source of the oil, it undoubtedly differs from Chinese wood oil, more particularly with regard to its powers of polymerisation and the iodine value is distinctly lower.

The following figures have been obtained by Chapman:

Source	Wakasa	Idzumo	?	Paulownia imperialis
Iodine value.	158.0	149.0	151.8	153.5
Sp. gr. 15/15°	0.9377	0.9400	0.9349	0.9351
Saponification value	195.2	193.4	196.3	193.5
Refractive index at 20°	1.5083	1.5052	1.5034	1.5050
Time of efflux at 15°, seconds	1230.0	1620.0		
Polymerisation, 2 hours at 250°	Soft.	Soft.	Very soft.	
Bromine thermal value (rise in degrees).				24.5

[1] 1914. 17, 38.
[2] *Oils, Fats and Waxes*, 5th Ed., 2, 82.
[3] *J. Coll. Sci. Imp. Univ. Tokyo*, 1906.
[4] *Bull. Imp. Inst.*, 1913, 13, 441.

Lewkowitsch[1] has contrasted the polymerising powers of Chinese and Japanese wood oils in the following table:

	Japanese tung oil	Chinese tung oil	
		No. 1	No. 2
Original oil..	0.93386	0.9412	0.9419
Heated rapidly in wide-mouthed flask to 213° (420°F.).	0.9649	0.9428	0.9432
Heated rapidly in wide-mouthed flask to 232° (450° F.).	0.9355	0.9445	0.9411
Heated rapidly in wide-mouthed flask to 250° (482° F.).	0.9477	0.9448	0.9504
Heated rapidly in wide-mouthed flash to 300° (572° F.).	....	0.9592	Solidified to hard jelly.
Heated rapidly in wide-mouthed flask to 310° (590° F.).	0.9553	0.9638	....
Heated rapidly in wide-mouthed flask to 320° (608° F.).	0.9650	0.9700	....
Heated rapidly in wide-mouthed flask to 330° (626° F.).	0.9694	Solidified to a jelly.	....
Heated rapidly in wide-mouthed flask to 340° (644° F.).	0.9760	....	....
Heated to 150° and kept there for 2 hours..........	Solidified to a soft jelly. 0.9477	0.9365 0.9363	0.9463

WALNUT OIL.

A. Fouchet[2] has extracted by means of cold petroleum ether a yellow oil from seeds of a cross between *Juglans niger* and *Juglans cinerea* the yield being 50%. The oil so obtained gave the following figures:

Sp. gr. at 12/4° C.. 0.925
Ref. index $(n)_D$ at 22° C 1.4765
Critical temperature of solution in absolute alcohol............... 78.5° C.
$|a|_D$.. ±0
Saponification value... 191.0
Acid value... 0.37
Iodine value... 151.0
Acetyl value... 11.0

The author states that the oil consisted mainly of the glycerides of stearic oleic, linoleic and linolenic acids, there being 70% of linoleic acid. *Juglans niger* is largely cultivated in North America, the oil from which is known as *pecan oil*.

The following figures were obtained by A. C. Deiler and G. S. Traps[3] for oil extracted from the seeds (kernels) by ether.

Sp. gr. at 15/15° C... 0.9184
Saponification value... 198.0
Iodine value (Hubl's method)................................... 106.0
Reichert-Meissl value... 2.2
Insoluble fatty acids + unsaponifiable......................... 93.4

ALIZARIN OIL, TURKEY-RED OIL.

W. Herbig takes advantage of the fact that the potassium salts of ricinoleic and sulphoricinoleic acids are largely soluble in cold acetone and the sodium salts only sparingly soluble, to effect a practically quantitative separation from the neutral oil. He proceeds as follows: From 2 to 5 grm. of

[1] *Oils, Fats and Waxes*, 5th Ed., 2, 84.
[2] *Bull. Sci. Pharmacol.*, 1912, 18, 529.
[3] *Amer. Chem. J.*, 1910, 43, 90.
[4] *Färber-Zeit.*, 25, 169 and 194.

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the oil, according to the water content determined by Fahrion's method[1] are neutralised with $N/1$ or $N/10$ alkali, evaporated to dryness on the water-bath and the residue dried by Fahrion's method (*loc. cit.*). The dried mass (which must not be overheated) is boiled with 4 successive portions (75 c.c. each) of anhydrous acetone, each extract being cooled with ice and decanted through a filter. The solution is evaporated, the residue of oil weighed and its acid and saponification values determined. The separated salts are readily soluble in hot water yielding a solution ranging from faint yellow to deep yellow ("monopol soap"). This solution is treated with boiling hydrochloric acid to liberate the combined sulphuric acid and fatty acids, the latter being subsequently extracted with ether and examined. From 66% ("monopol soap") to 77% (Turkey-red oil) of the total sulphuric acid was found in the salts insoluble in acetone.

The ratios between the percentages of acetone extract and fatty acids were: "monopol soap" 1.13; Turkey-red oils 1.45 and 1.78. The sum of water and total fat constituted about 90% of the samples of oil (84% in the case of "monopol soap"). This affords a practical sorting test.

CROTON OIL.

It is of interest to note[2] that this oil entirely loses its physiological properties when subjected to the process of hydrogenation.

BASSIA TALLOW.

Bassia Longifolia and Bassia Latifolia.—The seeds of Bassia longifolia and Bassia latifolia are very similar and are much confused not only on account of admixture in commercial samples but more especially because they are commonly known under the same names, such as "Mowrah," "Mohwrah," "Mahua" and "Illipe;" the latter name being also applied to a very large number of exotic fats and ceases to have any designative value.

The present revisers (see *Fatty Foods*, p. 183 *et seq.*) have endeavoured to draw some better line of distinction between the fats of this group and suggest that less confusion would arise if they were referred to as "Latifolia Fat" and "Longifolia Fat."

Bassia Longifolia.—Occurs in southern India *only*. The seeds are somewhat similar to those of *B. latifolia*, but as a general rule are slightly longer and narrower, but this does not hold true in every case.

The kernels which represent about ¾ of the weight of the seed contain some 55% of fat.

In all probability the fat from these seeds yielded the original "Illipe butter."

Bassia latifolia occurs mainly in central India—from western Bengal to Burma—but does not extend to southern India. The seeds are rather more

[1] *J. Soc. Chem. Ind.*, 1913, **32**, 1118.
[2] *Ber.*, 1909, **42**, 1546.

round and shorter than those of *B. longifolia* and larger than those of *B. butyracea*. As in the case of *B. longifolia* the kernel represents ¾ (or rather more) the weight of the seed and contains from 57–60% of fat.

Bassia Butyracea.—The seeds of *B. butyracea*, which occurs in the sub-Himalayan districts—from the Ganges to Bhutan—are very similar in appearance to *B. longifolia* and *B. latifolia* except that they are much smaller.

The fat obtained from the seeds is known as "Phulwa," the name "Phulwara" being applied to the seeds only. The seeds contain some ¾ of their weight of kernel, which kernel has a fat content of some 66%. This fat is one of the most common adulterants of Ghee and on this account has actually been given the name of "Ghee" in some text-books. The present revisers with a view to differentiating between the fats obtained from the three foregoing seeds examined samples of authentic origin and having extracted the fat themselves by means of petroleum ether, obtained the figures given in the following table:

Determination	Bassia latifolia	Bassia longifolia	Bassia butyracea
Ref. index at 40°C. (Zeiss butyro-refracto-meter.	47.7	49.3	47.8
Iodine value (Wijs)...............	59.4	62.6	42.6
Saponification value...............	192.2	189.8	188.2
Sp. gr., 99°/15C...............	0.8595	0.8624	....
Free fatty acids (as oleic)...............	24.6%	3.3%	8.74%
Unsaponifiable matter...............	....	....	1.36
Baryta value:			
(a) Total...............	263.0	258.2	257.3
(b) Insoluble...............	252.0	252.8	255.7
(c) Soluble...............	11.0	5.4	1.6
b—(200+c)...............	+41.0	+47.4	+54.1
Reichert-Meissl value...............	....	....	1.31
Polenske value...............	....	....	0.65

Shea butter or Karité butter is obtained from the seeds of *Butyrospermum* (or *Bassia*) *Parkii*, a tree largely grown in West Africa, French Soudan, etc. The general appearance of the seed is not unlike that of *B. longifolia*, *B. latifolia* and *B. butyrospermum*, though so very considerably larger in size as to render it impossible to be confused with these.

The whole seeds have a varying content of fat—amounting to 33 to 45% of its weight which is equal to 50–60% of the kernel, the latter being the portion usually imported.

Originally the fat found an outlet for the manufacture of soap and candles, but of late years, owing to improvements in the methods of deodourising and refining, its uses as an edible fat in the form of a lard substitute or pastry fat has been very considerable. The "stearine" has been utilised to a limited extent as a chocolate fat and the "oleine" for baking purposes.

One of the disadvantages of its use for edible purposes was at one time its large content of unsaponifiable matter—(5–9%)—but manufacturers have now learned how to select seeds giving the lowest yield of unsaponi-

fiable matter, and methods of removing a proportion of the latter have come into use. The figures in the following tables were obtained by the present revisers.

SHEA NUT OIL.

Determination	Usual limits	Typical specimen
M. p., °C., incipient fusion	29° to 32°	....
M. p. °C., complete fusion	37° to 42°	41.2°
Solidifying point, °C	25° to 30°	26.8°
Saponification value	180 to 190	186.9
Ref. index at 40° C. (Zeiss butyro-refractometer)	55.5 to 56.5	56.3
Iodine value (Wijs)	57 to 63	58.93
Sp. gr., 15°/15 C	....	....
Sp. gr., 99°/15 C	....	....
Free fatty acids (as oleic)	2 % upwards	8.29 %
Unsaponifiable matter	5 to 9 %	7.56 %
M. p. of fatty acids, °C	....	....

Determination	Shea nut "stearine"	Shea nut "oleine"
M. p., °C., incipient fusion	40.0°	....
M. p., °C., complete fusion	55.5°	....
Solidifying point, °C	34.2°	24.0°
Saponification value	179.7	181.6
Ref. index at 40° C. (Zeiss butyro-refractometer)	52.7	58.7
Iodine value	51.9	62.3
Free fatty acids	3.4 %	5.89 %
Unsaponifiable matter	6.25 %	7.72 %
Reichert-Meissl value	....	2.60
Polenske value	....	0.72

Bassia Toxisperma (Mimusops Djave), the seeds of which are commonly known as "Njave" or "Djave" being the "mahogany nuts" of the Gold Coast Colony.

The nuts are about 2½–3 in. long and 1¼ broad, having a bright polished mahogany coloured shell and a long oval hilum on one side. They are similar to, though rather larger and more pointed at the extremities than, Shea nuts. They contain about half their weight of a kernel in which there is 65 to 70% of fat.

As far as the present revisers are aware the fat has not been used for edible purposes and this is due to the fact that it usually contains traces of hydrocyanic acid produced by the enzymic decomposition of the non-fatty portion. It would be a comparatively easy matter to free the fat from this poison and in the event of no other unwholesome substance revealing itself, there seems to be no reason why this fat should not find a use for dietetic purposes.

The following figures have been obtained on a sample of the oil extracted from the seeds with petroleum ether by the present revisers.

Solidifying point	21.0°
Saponification	184.2
Ref. index at 40° C. (Zeiss scale)	51.8°
Iodine value (Wijs)	65.1
Sp. gr. 99½s	0.8578
Free fatty acids (as oleic), %	9.27
Unsaponifiable matter, %	3.86
M. p. of fatty acids	52.8°
Solidifying point of fatty acids	47.8°

Bassia Mottleyana, Nat. Ord. Sapotaceæ.

The seeds, which are also known as katio, katiau, ketzian and by various other names, are like those of *B. latifolia* but very much smaller. According to Brooks[1] the tree grows abundantly in the swamps of Sadong and Saribas Districts. This author states that they are at present of no commercial value, but are highly prized by the natives for cooking and other purposes.

The average figures of a Dyak prepared oil are given in the table below. This oil had a bright yellow colour, sweet taste and pleasant odour of almonds.

Brooks has found the kernels to contain 47.5% of oil, while another sample examined by the present revisers had a fat content of 56%, and the proportion of kernel amounted to 75% of the weight of the whole seed, 100 of which weighed 30 grm.

Brooks describes the Dyak prepared oil as having a pleasant odour of almonds, which neither the Imperial Institute nor the present revisers have found to be true of oils which were extracted from the seeds in the laboratory. The native prepared fat was, however, found to have a pronounced smell of almonds and this was investigated by the Imperial Institute who found no prussic acid, but proved the presence of benzaldehyde, which they suggest had been added for the purpose of flavouring or scenting the oil.

OIL FROM BASSIA MOTTLEYANA.

Description of sample	Dyak or native make			Extracted from seeds	
Observer	Brooks	Present revisers	Imperial Institute	Imperial Institute	Present revisers
Solidifying point.............	14.0	15.0	....	....	....
Acid value................	1.8	1.7	2.3	77.9	.13.8
Sp. gr., 15°/15°.............	0.917	0.9174	....	....	....
Sp. gr., 100°/15.5°..........	....	....	0.864	0.885	....
Iodine value...............	63.2	66.5	65.0	65.0	65.2
Ref. index................	53.4	....	....	....	52.3
Saponification value.........	189.5	188.9	191.5	191.0	192.1
Unsaponifiable matter.......	0.41	....	....	....	....
Reichert-Meissl value........	....	....	0.6	0.8	....
Titer test	....	....	36.3°	36.4°	....

BORNEO TALLOW.

During recent years very large quantities of Borneo tallow have found their way on to the European market in the form of cacao-butter substitutes, the fat being obtained from various kinds of *Shorea*, chiefly *Shorea stenoptera*, *Shorea ghysbertiana*, *Shorea aptera* and *Shorea robusta* as well as *Isoptera borneensis* and species of *Hopea*. The seeds are usually sent into this country under the name of "Pontianak illipé nuts," being distinguished by the prefix

[1] *Analyst,* 1909, 34, 207.

"large" or "small" this not conveying any botanical distinction, but being purely a commercial differentiation of the dimensions of the seed.

The cacao-butter substitute is commercially known as "green butter," but it must be carefully borne in mind that this name also includes the fat of a number of similar exotic nuts.

The fat obtained from certain of these seeds is so similar in physical and analytical properties to true cacao butter that the problem of distinguishing it from cacao butter is of the highest difficulty. Various tests have been put forward with the object of detecting its presence, one of the most important having been suggested by Halphen.[1] His test has been investigated by the present revisers[2] who not having found it altogether satisfactory, have modified it in the following manner: 1 grm. of the clear filtered fat is dissolved in 2 c.c. of a mixture of equal parts of carbon tetrachloride and petroleum ether (distilling below 40°), and 2 c.c. of this solution are placed in a test-tube about 6 in. long and ¼ in. in diameter. The tube is cooled in water and a solution of bromine in an equal volume of carbon tetrachloride added drop by drop, with constant shaking, until the colour of the bromine is permanent. The greatest care must be taken that only 1 drop in excess is allowed. The tube is then corked and allowed to stand. If, after the expiration of 15 minutes, the solution is perfectly clear, cacao butter is not present, or there is less than 10%. If the solution shows any turbidity, the presence of cacao butter is indicated, except in the case of one —somewhat rare—cacao butter substitute obtained from a species of Gutta nut. This one exception, however, does not give quite the same turbidity as cacao butter, and can easily be distinguished as described below.

The method can be made roughly quantitative by making mixtures of cacao butter and some solid fat of low iodine value (such as cocoanut oil or cocoanut "stearine" if an actual "green butter" is not to hand), and comparing the turbidities produced by these mixtures and the sample under examination.

After the turbidity has been compared, 2 c.c. of petroleum ether are added to the tubes, which, after mixing by inversion, are allowed to stand all night, when the cacao-butter turbidity settles out as a very fine canary-coloured precipitate, easily distinguished from the slight flocculent precipitate which "green butters" under these circumstances usually throw down. It is to be also noted that cacao butter is completely soluble in the carbon tetrachloride-petroleum-ether mixture in the strength given above, whereas "green butters" usually become turbid almost immediately, and on standing for 2 hours usually throw down a considerable precipitate. Care must therefore be taken that the solution used for the test is quite clear.

The fat mentioned above, which might possibly be mistaken for cacao butter, may be distinguished from true cacao butter as follows: The solution

[1] *J. Pharm. Chim.*, 1908, 28, 345.
[2] *Analyst*, 1913, 38, 201.

of the fat, after treatment with the bromine, is allowed to stand for 15 minutes, and the turbidity is then carefully examined by transmitted light. The turbidity due to cacao butter is absolutely non-flocculent, and any appearance of flocculent particles is characteristic of the other fat. If now to the brominated solution are added 2 c.c. of petroleum (fraction of motor spirit distilling between 90° and 100° C.) and the whole mixed, any turbidity due to cacao butter entirely dissolves, whilst the turbidity due to this other fat remains quite insoluble.

By this means 5% of this fat may be detected in admixture with 95% of cacao butter or "green butter." More than 10% of this fat produces such a heavy flocculent precipitate that it could not possibly be mistaken.

Since this test was put.forward difficulties have arisen in that many of the commercial products now often contain proportions of *hydrogenised* fats which considerably mask, and in some cases, vitiate the test.

ERRATA AND ADDENDA TO VOL. II.

Page 91, line 4 from bottom; H. Meyer and D. Beer[1] state that stearic and hypogæic acids are not present and that they have found the fatty acid of high melting point isolated by Hehner and Mitchell to be a mixture of arachidic and lignoceric acids.

Page 105, the section on apricot-kernel oil should commence "see also pp. 44 and 69."

Page 119, footnote 2, add "Chemist and Druggist, 1901, Sept. 21.

Page 176, cacao-butter section should commence "see also this volume, p. 71, and Vol. VI, p. 715.

Page 177, line 10, "30" should be "30°."

Page 180, at head of right-hand column, the o in front of degree sign should be deleted.

Page 188, at end of second paragraph there should be a reference to a new footnote as follows:

"Elsdon (*Analyst*, 1912, 38, 8) by the method of alcoholysis concludes that the composition of the mixed fatty acids is approximately as follows: caproic acid, 2%; caprylic acid, 9%; capric acid, 10%; lauric acid, 45%; myristic acid, 20%; palmitic acid, 7%; stearic acid, 5%; oleic acid, 2%."

Page 194, footnote 2 should be at the bottom of page 193, and the refer ence figure 2 should be inserted in the text after "Mitchell" in line 8 from bottom of page 193.

Page 217, line 9 from bottom, the second "or" should read "of."

Page 226, last line, "191.4" should read "196.2."

Page 231, in columns 8 and 14, the figures 5.60 and 65.92 opposite "R.-M. value" should be opposite "Reichert value."

Page 246, the words in brackets, lines 10 to 7 from bottom, should have been a footnote. There should be a comma after "410" and the semicolon after the second bracket should be omitted.

Page 252, the first footnote should be numbered 1.

Page 257, insert a new paragraph:

Paraffin wax, if present to the amount of 3% or more, can be easily detected by saponifying 5 grm. of the sample with alcoholic potassium hydroxide as in determining the saponification value and keeping the liquid hot on the water-bath in a corked flask. The paraffin will be seen floating on the surface of the liquid or adhering in small globules to the sides of the flask and the solution when diluted with hot water will be turbid. Genuine beeswax gives a clear solution

[1] Monatsh., 1913, 34, 1195.

BUTTER FAT.

By CECIL REVIS and E. R. BOLTON.

COMPOSITION OF BUTTER FAT.

Siegfield[1] has carried out some work on the acids present in butter fat and is of the opinion that the non-volatile acids consist of oleic, palmitic and myristic acids, a considerable quantity of the latter being sometimes present. Stearic acid was not found. The volatile acids consist chiefly of butyric, caproic and caprylic acids in very variable proportions, while the volatile insoluble acids contain a small quantity of caprylic acid and probably traces of palmitic and myristic acids. According to v. Fodor[2] caproic acid is the normal acid and not the isobutylacetic acid; this is confirmed by Smedley,[3] but the latter investigator finds 10 to 15% of stearic acid which is very improbable.

Content of Volatile Fatty Acids, Soluble and Insoluble in Water (Compare Vol. II, p. 283).—The Reichert-Meissl-Polenske method has been extended by the writers to work in connection with the method of Kirschner. The full process will be found under *Margarine*, page 166. As was pointed out by Kirschner and confirmed by the writers, this latter value is practically a measure of the butyric acid present and consequently gives a more sensitive indication for the detection of coconut oil than the Polenske value alone. The writers have suggested the following comparative values:

Kirschner value	Polenske value
20	1.6
22	2.1
24	2.6
26	3.2

These figures have been confirmed by Cranfield,[4] who has determined the R. M. Pol. & K. values for a large number of butters.

A variation of 1.0 must be allowed either way in the Polenske value corresponding to any particular Kirschner value, the addition of less than 5% of coconut oil causing the Polenske value to fall outside this limit.

A long and searching investigation of the variations in the Reichert-Meissl and Polenske figures for the butter fat of single cows over the whole period of lactation has been carried out by Beerbohm.[5] It appears that,

[1] *Zeit. Unters. Nahr. Genussm.* 1912, 24, 45.
[2] *Ibid.*, 1913, 26, 641.
[3] *Biochem. J.*, 1912, 6, 451.
[4] *Analyst*, 1915, 40, 439.
[5] *Milch. Zentr.*, 1913, 42, 513.

in general, the Reichert-Meissl figure falls during lactation, whilst the Polenske figure rises. The curves and tables given are very interesting, but are too lengthy for reproduction here.

According to Sunberg,[1] the percentage of coconut oil may be calculated from the tables given by Polenske[2] by taking the percentage there given, as percentages referred to the actual butter fat concurrently present and then calculating the percentage on the mixed fat. For instance, if the Polenske figure obtained gives from Polenske's tables 27%, then the actual percentage present in the mixture would be given by $\dfrac{27 \times 100}{100 + 27} = 21.5\%$.

The question of the likelihood of obtaining butter fat apparently adulterated with coconut oil, from cows fed on coconut-oil cake, has been investigated by Ledent,[3] whose results appear to show that the butter fat in such cases does give indications of the presence of coconut oil. This opinion has been confirmed by Barthel and Soden,[4] who show that it is not only the case with coconut-oil cake, but also with that of beet-root leaves. The writers are of the opinion that while this may occur occasionally in practice, it has not come under their notice in samples of butter representing the chief supplies of the English market. Should this adulteration with coconut oil be suspected, it could be confirmed by the phytosteryl acetate test, which would give positive results for the presence of phytosterol if the recrystallisation is carried out sufficiently often.

The Method of Avé Lallemant (Compare Vol. II, p. 288).—This method has been used by the writers since the time of its publication and there seems little doubt as to its value.

The following are a selection of figures obtained by them with different butters:

Butter	Total Ba	Insoluble Ba	Soluble Ba	Difference	R.M. No.	Polenske No.	Kirschner No.
Danish...............	315.90	252.95	62.95	− 10.00	29.7	2.7	24.2
	308.59	251.18	57.41	− 6.23	30.6	2.7	23.8
	310.38	253.31	57.07	− 3.76	30.2	1.9	23.8
	314.38	254.19	60.19	− 6.00	31.8	2.9	22.8
	312.03	254.30	57.73	− 3.43	30.1	1.8	21.4
	312.18	252.98	59.20	− 6.22	30.4	2.4	21.4
	317.66	256.04	61.62	− 5.58	31.8	3.0	20.9
	316.19	254.95	61.24	− 6.29	30.9	2.9	....'......
	317.52	255.90	61.62	− 5.72	29.1	3.0	....
	312.78	252.24	60.54	− 8.30	31.4	2.3	....
English...............	313.16	253.18	59.98	− 6.80	30.1	2.3	24.6
	312.71	252.98	59.73	− 6.75	29.8	2.4	21.9
	312.04	253.57	58.47	− 4.90	30.1	2.5	21.9
	314.38	255.35	59.03	− 3.68	28.3	2.1	20.1
	312.26	251.61	60.65	− 9.04	31.4	2.4	22.9
	312.88	255.42	57.46	− 2.04	29.8	2.4	....
	313.46	254.44	59.02	− 4.58	28.5	2.4	....

[1] *Zeit. Unters. Nahr. Genussm.*, 1913, 26, 422.
[2] *Arbeit Kaiserlich Gesundheitsamte*, 1904, 5, 45.
[3] *Bull. Soc. Chim. Belge.*, 1913, 27, 325.
[4] *Zeit. Unters. Nahr. Genussm.*, 1914, 27, 439.

Butter	Total Ba	Insoluble Ba	Soluble Ba	Difference	R.M. No.	Polenske No.	Kirschner No.
New Zealand.........	309.78	251.40	58.38	− 6.98	30.5	2.2	23.1
	316.90	251.86	65.04	−13.18	32.7	2.7	24.7
	311.11	252.03	59.08	− 7.05	31.8	2.2	22.0
	317.59	253.87	63.72	− 9.85	29.6	3.3	....
	313.41	252.91	60.50	− 7.59	32.4	2.7	....
	318.70	254.50	64.20	− 9.70	32.2	3.0	....
	316.25	254.15	62.10	− 7.95	32.4	2.6	....
Irish...............	315.83	256.23	59.60	− 3.37	32.2	2.8	....
	314.68	254.03	60.65	− 6.62	31.4	2.5	....
	311.41	254.24	57.17	− 2.93	28.1	2.1	....
	309.05	251.55	57.50	− 5.95	27.4	2.3	19.2
Normandy..........	316.43	253.90	62.53	− 8.63	32.4	3.2	....
	316.26	252.52	63.74	−11.22	31.9	3.0	....
	311.94	254.95	56.99	− 2.04	28.8	2.1	...,......
	316.96	256.73	60.23	− 3.50	31.4	2.9	....
Probably adulterated..	312.75	258.17	54.58	+ 3.59	28.5	2.3	....
	307.22	256.30	50.92	+ 5.38	26.6	1.8	....
	309.13	257.89	51.24	+ 6.65	27.1	2.1	....
	303.06	255.48	47.58	+ 7.90	24.6	1.7	....

The above figures illustrate the type of result obtained. There is a great similarity in butter from different sources, and in the case of butters arriving from a known source, the method is of the greatest value. In many cases in which a positive result was obtained indirect evidence was forthcoming to support the analytical data.

It is necessary again to point out that the greatest care must be exercised in the analytical technique, more particularly in the original saponification titration, which must be obtained to the nearest half drop.

Qualitative Tests.

The Foam Test (Compare Vol. II, p. 299).—This test, originally supposed to be of use in distinguishing between butter and margarine, has lost much of its value on account of the additions now made to margarine in order to bring about "browning" and "foaming" which are considered so essential in ordinary culinary operations. These compounds generally consist of some compound of casein together with sugar, and egg yolk is also used for the some purpose.

Halphen's Test.—This test has been improved by Gastaldi[1] by substituting pyridine for the amyl alcohol employed.

The modified test is carried out as follows: To 5 c.c. of the oil add 1 drop of pyridine, shake well and after adding 4 c.c. of carbon disulphide, containing 1% sulphur, heat for 20 minutes in the water-bath, the tubes being closely stoppered. The writers can confirm the value and greater sensitiveness of the test; they find it possible to detect at least 0.2% of ordinary cottonseed oil products. It must be remembered that hydrogenation (see pages 122 and 173) partly destroys the chromogenetic substance responsible for the

[1] Abs. *J. Chem. Soc. Ind.*, 1912, 31, 934.

reaction, and for this reason the greatest possible sensitiveness that can be obtained for this test is desirable.

Utz has suggested the use of pentachlorethane (b. p. 159° C.) as solvent for the sulphur. The tubes can then be heated at a temperature nearly that of the boiling point of the solvent. It is stated that the test is rendered more delicate, but the writers have not as yet had experience of the method. (See Cottonseed Oil, p. 135.)

Water (Compare Vol. II, p. 304).—There has been a marked tendency of late years for the water content of butter to approximate to 16%. This is shown by the following successive figures obtained for various butters:

Danish.
15.8, 14.9, 16.2, 15.6, 15.8, 15.9, 15.7, 15.7, 15.7, 15.9, 15.1, 15.8, 15.5, 14.6, 15.0, 15.3, 15.4.
Blend 1.
15.0, 14.0, 15.3, 15.2, 14.7, 15.3, 15.9, 15.3, 14.7, 15.7, 15.3, 15.9, 15.6, 15.4, 15.7.
Blend 2.
15.0, 15.7, 15.2, 16.1, 15.8, 16.4, 15.9, 15.4, 16.7, 15.9, 16.5, 15.9, 14.6, 15.9, 15.8, 14.9, 15.9.
English (country made).
14.6, 14.9, 14.1, 14.2, 14.4, 14.5, 13.5, 14.7, 14.3, 15.4, 15.3, 15.4, 14.2, 13.8, 14.0, 14.2.
Irish.
14.0, 14.0, 13.7, 14.4, 13.8, 13.6, 13.9, 13.6.

The difference between the last two which do not compete on so keen a market, and the first three which do so compete, is very noticeable.

A few methods for the rapid estimation of water in butter have been devised for factory work, but it is probable that the ordinary direct drying method is really the simplest, though for obvious reasons the time required is not possible in factories where blending is going on, and the percentage of water is required while the butter is passing the blenders.

The method of Patrick (Vol. II, p. 306) is probably the most expeditious; it is quite satisfactory, but requires some little skill and attention to hit the exact point when all the water has gone and decomposition of the curd has not commenced.

For this reason distillation methods have been introduced which are rapid and quite accurate enough for the purpose.

(1) **Gray's Method** (*U. S. Dept. of Agric., Bureau of Animal Industry,* Circ. 100).

Apparatus.—The apparatus required for the test is as follows:

Balance.—Sensitive to 0.025 grm.

Pipette.—For measuring 6 c.c.

Paper.—Parchment, 5 by 5 in.; must be perfectly dry.

Special Apparatus.—As shown in figure. Referring to Fig. 6, *A* is a flask of capacity of a little over 70 c.c. *C* is a graduated tube, which is connected with flask *A* by means of a rubber stopper. *F* is a glass stopper ground into the tube *C*. The tube *C* is graduated after this glass stopper *F* has been ground in, the zero mark being the end of the stopper. Each mark of the graduation represents 0.02 c.c., or when a 10-grm. sample of butter is used each mark represents 0.2% of water. *E* is a glass condensing

jacket connected to the graduated tube *C* by rubber stopper *D*, as shown in the figure.
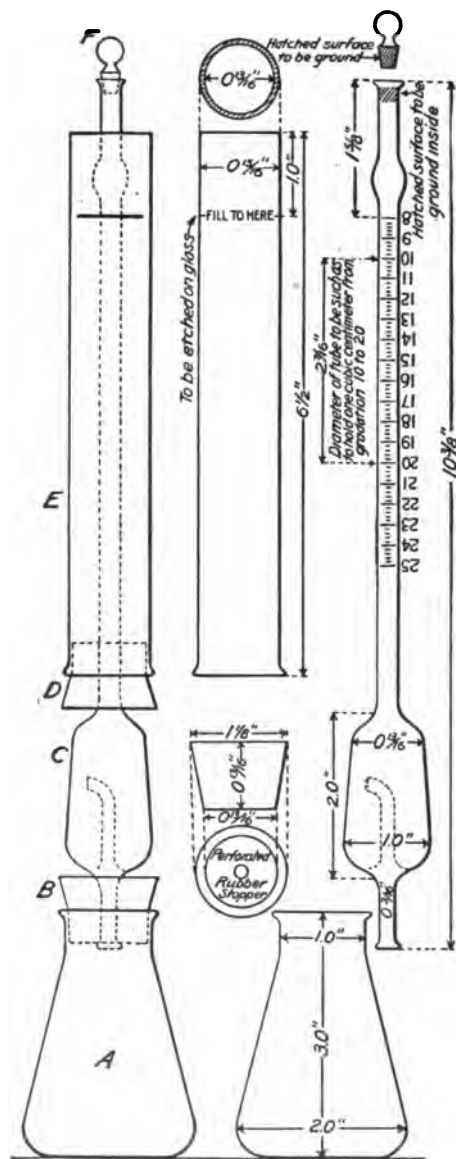
FIG. 6.

Amyl Reagent.—A mixture of amyl acetate (5 parts) and amyl valerate (1 part). Must be free from water soluble impurities in order to give accurate results.

Method.

Preparing the Sample.—The sample of butter is placed in a suitable container (1 pint jar or metal cup will be satisfactory), and the latter immersed in water at about 100° F. The butter is stirred with a spatula or spoon until it has the consistency of thick cream and no free water can be seen. Samples of butter should not be left standing in open containers any length of time before making the water determination, as some of the water will evaporate and the percentage of water found finally will be too low.

Weighing the Sample.—Place on each pan of the balance one sheet of parchment paper and balance accurately. Place the 10 grm. weight on one pan and balance again by placing butter on the parchment paper on the opposite pan, placing the sample as near the centre of the paper as possible.

When exactly 10 grm. are weighed out remove the sample from the pan, and fold it in the parchment paper in such a way that the paper and butter may be slipped into flask A. Add 6 c.c. of the amyl reagent to the butter in the flask, connect the apparatus as shown in the figure and fill the condensing jacket E with cool water to within 1 in. of the top. Remove the stopper F.

Place the apparatus over the flame of the burner, applying heat to the bottom of the flask A. In a short time the butter will melt, running from the parchment paper into the amyl reagent. The water in the sample then boils and passes as steam into the tube C, where it is condensed and trapped. Watch the condensation in the graduated part of the tube C, and do not let the steam get higher than the 15% mark. If it goes higher than this, remove the flame, as there is danger of water being lost. If there is any indication of the mixture in the flask A foaming over, remove the flame. Foaming is usually prevented by 6 c.c. of amyl reagent, but some samples of butter, especially those of high moisture, require a trifle more than 6 c.c. In case of continued foaming, allow the mixture in the flask to cool, and add about 2 c.c. of the amyl reagent, and continue heating. After the water in the sample has boiled out, the temperature rises and the amyl reagent boils, driving the last traces of water and water-vapour from the flask and bottom of the stopper. Some of the amyl reagent is carried into the tube C with the steam, and some is boiled over after the water has been driven off. This amyl reagent in the tube is no disadvantage. The time required to expel all the water from the sample is not less than 5 minutes and with most samples need not be more than 8 minutes. When the mixture in the flask becomes brown and all the crackling in boiling ceases, it is safe to conclude that all water has been driven from the flask. Disconnect the flask A from the stopper B, place the glass stopper F in the tube C, giving it a slight turn to ensure its being held firmly, invert the tube C, first being sure that the mouth of the small tube inside the bulb is held up-

wards; pour the water from the condensing jacket E, after which the jacket may be removed. When the tube C is inverted the water and amyl reagent flow into the graduated part of the tube. To separate these and to get the last traces of water into the graduated part, the tube C is held with the bulb in the palm of the hand and the stoppered end away from the body, raised to a horizontal position, and swung at arm's length sharply down to the side. This is repeated a number of times until the dividing line between the water and the amyl reagent is very distinct and no amyl reagent can be seen with the water and *vice versa*. The tube should then be held a short time with the stoppered end downwards and the amyl reagent in the bulb of the tube agitated in order to rinse down any water that may be adhering to the sides of the bulb. The reading should not be taken until the tube and its contents have cooled so that very little warmth is felt. The water is in the bottom of the tube, and when a 10-grm. sample is taken the percentage may be read directly. Read the lower part of the meniscus.

The following method has been devised to estimate **fat and salt in butter,** particularly in creameries.[1]

Estimation of Fat: Apparatus Required.—A centrifuge.

A special separating funnel.

A balance which is sensitive to 0.01 grm. (A torsion balance such as is used in the moisture test is satisfactory if it is in good condition.)

An accurate set of metric weights.

A 10 c.c. graduated glass cylinder.

A 100 c.c. glass beaker.

Special Separating Funnel.—This is essentially a separating funnel with a capillary stem. The capacity of the funnel should be about 75 c.c. and its weight when empty should not exceed 70 grm. The stopper may be dispensed with if desired. It is a convenience in the final weighing, but not a necessity. Fig. 7 shows the form and dimensions of the funnel.

Special Socket.—This is a double socket for holding the above funnel while centrifuging, and is made of heavy sheet copper with hangers of steel. Each socket will hold 2 funnels. The cut shows the construction and dimensions. It differs in no material way from the socket ordinarily used on the Babcock centrifuge, except for the opening in the side. If the dimensions given fail to fit the centrifuge at hand, they may be changed to suit so long as the dimensions of the barrels are not altered. Care must be taken that the capillary stem of the funnel does not project far enough through the hole in the socket to strike against the side of the centrifuge when being whirled. It is best to fit a disc of rubber to the bottom of the socket.

Sampling the Butter.—In estimating fat in butter, great care must be taken in securing a representative sample and in preparing this for the test.

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[1] Shaw, *U. S. Dept. of Agric. Bureau of Animal Industry,* Circ. 202, May 20, 1912. (Compare Vol. II, p. 306.)

Errors introduced by improper sampling are far greater than those in the actual test.

Samples are best taken with a butter trier, and one should always take several plugs from different parts of the tub or churn. These are placed in a suitable container, such as a 1-pint preserve jar or a cup, which is placed in water at about 100° F. The sample is then mixed with a spatula or spoon until about the consistency of thick cream. The sample must not be left
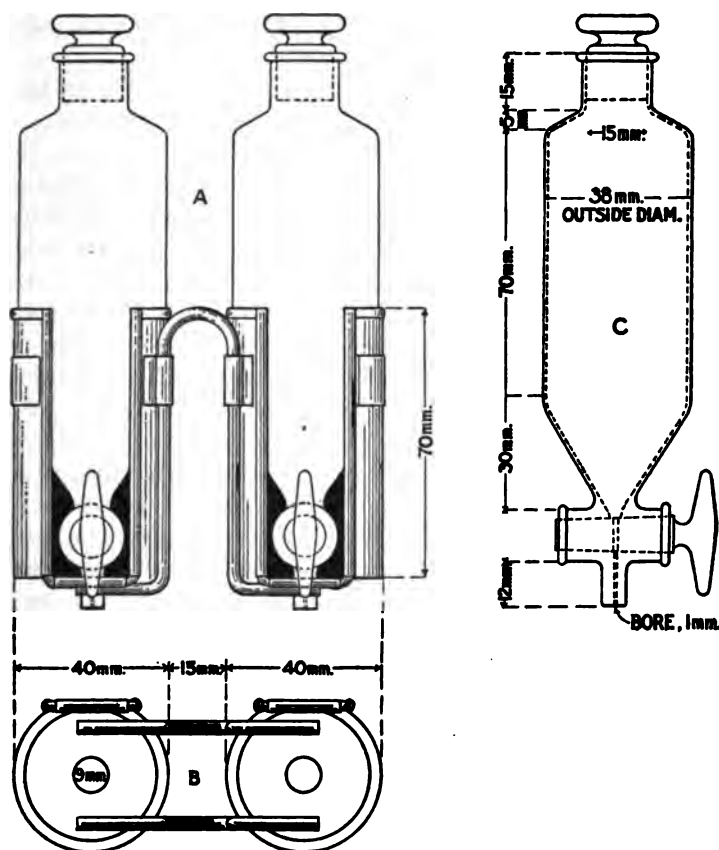
FIG. 7.—The special apparatus for estimating fat and salt in butter. A, the socket with funnels in position; B; view of socket from below; C, the separating funnel with capillary stem. (Reduced one-half.)

any length of time in open containers, since some of the moisture will evaporate. Should the sample be kept for any reason for a day or two before it is mixed, it should be placed in warm water (with the cover on the container) until melted, and then cooled while being previously shaken until it solidifies. The reason for this is that on standing some of the water will ooze out and

cannot be reincorporated except by emulsifying and cooling while in this condition. Too much stress cannot be laid on careful sampling and mixing the sample, for upon this the accuracy very largely rests.

Estimating the Fat.—It will be found more economical in some cases if 4 or multiples of 4 estimations are made at once. In this case the 2 double sockets will balance when placed opposite in the centrifuge. If but 1 or 2 estimations are made it will be necessary to balance the centrifuge by putting weights in the opposite socket. The weigh of the clean, dry separating funnel must first be ascertained. This weight once found will suffice for all estimations made with that particular funnel.

I. *Weighing the Charge.*—Counterpoise the small beaker on the balance and carefully weigh out 20 grm. of the sample mixed as directed.

II. *Transferring the Charge to the Separating Funnel.*—Place the beaker containing the charge on a radiator or steam pipe until the butter is melted. (This may also be accomplished by adding a small quantity of boiling water.) Next pour the charge into the funnel, which must be maintained in an upright position, and no part of the charge lost in transferring. With a fine stream of hot water rinse down the sides of the beaker and pour the rinsings into the funnel. Repeat this, using not more than a teaspoonful of water at a time until the funnel is full to within $\frac{1}{4}$ in. of the shoulder. The rinsing can be done very conveniently with the arrangement on many steam centrifuges for filling the Babcock test-bottles, *i.e.*, the rubber tube ending in a glass or metal point and connected with a water tank heated by steam. The point must be fine, however. Should it be larger than $\frac{3}{16}$ in. it can be replaced with the tip of a small oil can. Should this arrangement not be at hand one can easily be improvised from a tin can, a rubber tube and an oil-can tip. In transferring the melted butter and rinsings the last drop may be prevented from running down the outside of the beaker by touching the lip of the beaker on the neck of the separating funnel.

III. *Centrifuging.*—Insert the separating funnel in the special socket, allowing the stem to project through the hole in the bottom and the handle of the stopcock through the open side. (Caution: The socket must always be placed in the centrifuge with the open side facing the direction in which the wheel revolves. This is very important, for if the opening faces the reverse direction the stopcock will be thrown out and broken.) Whirl 1 minute at the same speed used in testing milk on the Babcock method. The centrifuge must be kept warm.

IV. *Removing the Water.*—Remove the separating funnel from the socket and allow the water to flow through the stopcock until the fat (or curd) is within $\frac{1}{8}$ in. of the stopcock. In this and subsequent operations care must be taken that the stopcock does not stick. It must always be under control, and it is best to give it frequent slight movements when the water or acid is running through it to be sure that this control is maintained,

otherwise it may stick at the critical moment and the estimation be lost. Most of the salt and part of the curd are taken out by the water. The remainder of the curd and all of the fat stay in the funnel.

V. *Dissolving the Curd.*—Measure out 9 c.c. of cold water (preferably distilled) into the beaker with the glass measure. Add to this 11 c.c. of sulphuric acid (sp. gr. 1.82–1.83) and mix by gently shaking. While still very hot add the mixture to the contents of the separating funnel. Now dissolve the curd by giving the funnel a circular motion with the hand grasping the neck. Centrifuge 1 minute, as before. Draw off the acid solution until the fat layer is within ¼ in. from the stopcock and repeat the operations in this paragraph.

VI. *Freeing the Fat from the Acid Solution.*—The fat will now be in a clear transparent layer free from curd, and the solution below it will be practically colourless. To separate these, draw off the latter until the fat nearly reaches the stopcock, and centrifuge another minute. Allow the fat to descend through the stopcock until it just reaches the end of the capillary stem. This last step offers no difficulties, provided the stopcock is kept in control, but it requires care.

VII. *Estimating the Percentage of Fat.*—Carefully dry the separating funnel on the outside with a clean soft towel and weigh it. The weight thus obtained minus the weight of the empty funnel represents the weight of butter fat in 20 grm. of the sample. The percentage is obtained by dividing this weight by 2 and multiplying by 10.

Sometimes it is possible to obtain a clear layer of fat with but one addition of acid, but in the majority of cases it will be found necessary to add it a second time, as directed. The proportion of acid and water selected is the outcome of a number of experiments, and is the one which gives the best results. The test for fat alone involves 4 centrifugings of 1 minute each. The centrifuge should be kept warm and the contents of the funnel in a melted state when the acid is added. The time consumed should not be much longer than in testing cream by the Babcock test, and the operations involved are simple. No difficulty has been experienced in obtaining a clear fat. Occasionally a slight emulsion appears at the bottom of the fat layer when the latter is drawn into the stem. This is so small in amount that it does not seem to affect the accuracy of the test to any considerable extent. The emulsion should be weighed as fat and considered as such.

Cleaning the Separating Funnels.—The separating funnels should be washed after each estimation, but it is not necessary to dry them before use providing their weight, when clean and dry, has been found. The cleaning is easily done with hot water and either soap or cleansing powder. They should be well rinsed with clean water and drained.

11

Estimation of Salt.

Additional Apparatus Required.—A 50 c.c. burette graduated to 0.1 c.c.
A 250 c.c. volumetric flask.
A 25 c.c. pipette.
A 250 c.c. beaker or white cup.

Chemicals Required.—An aqueous silver-nitrate solution containing 14.525 grm. pure silver nitrate per litre and a 10% aqueous solution of potassium chromate.

Method.—To determine the percentage of salt the wash water, obtained, as previously directed in Paragraph IV, is allowed to run into the 250 c.c. flask, and the operations in Paragraph IV conducted 3 times instead of but once, the water each time being allowed to run into the flask.

After the washings have become cool the flask is filled to the mark with cold water and the contents mixed. 25 c.c., which represent 2 grm. of the original sample, are then measured with the pipette into the beaker or cup and titrated with the silver-nitrate solution from the burette, using 2 or 3 drops of the potassium chromate solution as the indicator. The first appearance of a permanent red is the end point. The silver-nitrate solution is of such strength that 2 c.c. represent 1% of salt if a 1-grm. charge is used.

In the above test where 2 grm. are represented $\left(\dfrac{25}{250} \times 20\right)$ the number of c.c. divided by 4 gives the percentage of salt in the original sample. As an example, if the burette reading showed that 10.6 c.c. of the silver-nitrate solution were consumed in reaching the end point, then 10.6 divided by 4, or 2.65, would be the percentage of salt in that particular sample.

Estimating the Percentage of Curd.

If the moisture is determined in a separate charge by one of the reliable methods, the percentage of curd may be found by subtracting the sum of fat, salt, and moisture from 100.

Benzoic Acid (Compare Vol. II, p. 311).—Hinks[1] has devised the following method of detecting and estimating benzoic acid (and incidentally salicylic acid) in milk products.

10–20 grm. of cream are heated with an equal volume of concentrated hydrochloric acid until the curd is completely dissolved and the mixture is cooled and shaken with 25 c.c. of normal methylated ether and petroleum spirit (1 : 2). The ethereal layer is separated, and 1 drop of ammonia (0.880) added and then 5 c.c. of water. The mixture is shaken, the aqueous layer separated, heated for a few minutes on a water-bath to expel ammonia and then tested for benzoic acid, in the usual manner, with ferric chloride. It is probably advisable to add a trace of acetic acid before the ferric chloride,

[1] *Analyst*, 1914, 38, 555.

in order to ensure against alkalinity in the test solution, and in order to be certain that the ferric chloride solution is neutral, ammonia should be added to the freshly prepared solution till the iron precipitates, the solution filtered and the filtrate used for the test.

Hinks has shown that on adding ammonia to the ethereal extract (before the addition of water) a precipitate of ammonium benzoate appears, whilst in the case of pure milk, no effect, or only a slight opalescence is produced, and that the test is very delicate for benzoic acid. It is probable that it is in no way characteristic of benzoic acid but that other organic acids, probably lactic acid, would show a similar pecipitate, for which reason, while note should be taken of this precipitate the result should be substantiated by the ferric chloride test.

The method is made quantitative by dissolving the cream as before, using a reflux condenser, the cooled solution being extracted 3 times with 20 c.c. of a mixture of equal parts of methylated and petroleum ethers. The mixed ethereal extracts are made alkaline with ammonia, 10 c.c. of water added and the mixture shaken, and the aqueous layer separated. This process is repeated twice more, adding more ammonia if necessary. The mixed aqueous extracts are made acid with hydrochloric acid and again extracted 3 times with 20 c.c. of mixed ethers. The combined ethereal extracts are allowed to evaporate spontaneously and the residue dried in a desiccator till constant in weight (about 24 hours). The residue is then heated at 100° for 1–2 hours and again weighed. The difference gives the benzoic acid in the original quantity of cream taken.

The method gives excellent results.

Butter should be shaken out violently with sufficient of a 1% solution of sodium bicarbonate, and the aqueous layer, after separation, boiled with hydrochloric acid and extracted with ether.

Cinnamic Acid.—This substance appears to be used occasionally as a preservative. In order to detect its presence the preservative is extracted, either as described under benzoic and salicylic acids (Vol. VIII, p. 190) up to the point of extracting the ether with barium hydroxide, or by Hinks' method (page 162). In either case the ether is extracted with ammonia, the aqueous layer evaporated to dryness, and the residue heated to boiling with 5 c.c. of dilute chromic acid solution (1 part dilute sulphuric acid (1 : 3) saturated with potassium dichromate and 7 parts water), in a covered crucible. The crucible is then cooled without opening and when cold the odour of benzaldehyde is at once noticed on removing the lid if so small a quantity as 0.2% of cinnamic acid is present in the original cream.

Phytosteryl Acetate Test (Compare Vol. II, p. 301).—The use of hardened vegetable fats has necessarily brought this test into greater prominence and utility, as it may be, in certain cases, the only method by which a hardened vegetable fat can be detected in admixture with animal fats.

The method of separating the sterols has been simplified by the use of digitonin which with these substances forms compounds almost insoluble in alcohol, and from which the original sterols are easily regenerated.

The method of applying the digitonin test first devised by Marcusson and Schilling[1] is given on page 118.

The method as thus devised gives difficulty sometimes as the digitonides form emulsions with the fat, and in any case it is only applicable if the sterols are present in the free state. It has been objected to by Klostermann[2] on the ground that esters of the sterols may be present and these are not precipitated by digitonin. He proposes to saponify the fat (100 grm.) with alcoholic potash in the ordinary way, and to dilute the saponified mass with water, acidify and extract the fatty acids and sterols with 250 c.c. of ether. The ether is washed with water and 250 c.c. of petroleum ether and 25 grm. sodium chloride are added. The water which separates is run off and the ether filtered through cotton wool. The filtrate is heated with 1 grm. of digitonin dissolved in 20 c.c. of 90% alcohol, and the crystalline precipitate which forms filtered after 15 minutes and washed free of oil with ether. This fat-free residue is then boiled with 20–30 c.c. of acetic anhydride, evaporated to dryness, dissolved in 50 c.c. of alcohol, and 25 c.c. of water gradually added. The precipitate is filtered off, washed with 70 c.c. alcohol, and recrystallised from 90% alcohol, in the usual way.

It is easier (particularly if more than 100 grm. of fat be used) to employ the method given by the writers (Vol. II) in which the fat is boiled out first with alcohol. The alcoholic extract is saponified once only, the fatty acids liberated, dissolved in ether, washed and treated direct with alcoholic digitonin solution (0.2 grm. digitonin per 100 grm. of fat). The digitonides are filtered and washed with ether to remove any traces of oil, dried and treated with acetic anhydride in the usual way, in an evaporating basin or stoppered tube (5 c.c. of acetic anhydride for 50 grm. of fat). The acetic anhydride is evaporated off and the residue taken up with absolute alcohol, and boiled if necessary with recently ignited animal charcoal (fine powder), filtered, evaporated to dryness and the residue recrystallised from 90% alcohol. The precipitation of the acetates from alcoholic solution by water, previous to final crystallisation as suggested by Klostermann (see *supra*) is not to be recommended, as the resultant liquid filters in some cases with great slowness. As it is generally necessary to crystallise the acetates 4 to 5 times, very small quantities of alcohol must be used for the recrystallisations and very small test-tubes should be employed. The crystals are filtered off in a very small funnel, having a glass bead fitting the neck. As each crop is thus filtered it is washed with 2 to 3 drops of 70% alcohol and the bead lifted and the crystals washed into a fresh tube with 1 to 2 c.c. of boiling 90% alcohol. The crystals are dissolved by heating and again allowed to separate. By thus avoiding

[1] *Chem. Zeit.*, 1913, 37, 1001.
[2] *Zeit. Unters. Nahr. Genussm.*, 1913, 26, 443.

filter paper, etc., no difficulty will be found in carrying even small quantities to 4 to 5 crystallisations.

A very small quantity of the crystals is placed on a porous tile and the melting point determined. Cholesteryl acetate melts at 113° C. (corr.), and phytosteryl acetate at from 125°–133° C. If the melting point of the fourth crystallisation be above 116° C. phytosterol may be assumed to be present in the original mixture.

Rancidity (Compare Vol. II, p. 313).—Recent investigations have added little to the knowledge of the causes of rancidity.

In the case of butter, it is necessary to distinguish two different types of rancidity which occur in practice. (1) The rapid change which takes place in butter after it has been placed on the market, particularly after it has been removed from cold storage, and is probably due entirely to the effect of light, possibly aided by the action of moulds. The change is confined to the outer layers of the butter. (2) The slow deterioration and loss of flavour which takes place when butter is kept in cold storage.

Investigation shows that sweet cream butter deteriorates much more rapidly than butter made from properly ripened cream; the lactic acid would appear to act as a preservative.

In spite of the fact that any lipolytic action on the fat appears to be negatived by the work of Rahn, Brown and Smith it is difficult to say that the deterioration in taste and rancid flavour may not be due to traces of free fatty acids such as cannot be actually estimated.

A most interesting investigation into the deterioration of storage butter has been made by Rogers and others[1] in which, amongst other possible factors, the action of small quantities of metals (particularly iron and copper) in producing deterioration has been investigated. As both these metals can easily be introduced into butter in minute quantities during making, and it is shown that they do produce decided deterioration, there appear to be grounds for attributing at least some of the loss of flavour to this cause. It is not improbable that the more rapid development of rancidity after removal from cold storage may be due to the preliminary stages having been so induced during storage.

The original bulletin should be consulted by those who are interested in the subject.

In connection with the general subject of faults in butter the following may be of interest and guidance to the analyst:

(1) A yeasty taste in butter may arise from repeated oversouring of the starter when yeasts develop which impart this flavour. Careless washing also intensifies this.[2]

(2) Lipolytic action may take place if starters are carelessly prepared,

[1] *U. S. Dept. of Agric., Bureau of Animal Industry, Bulletin* 162, April, 1913.
[2] Rosengren, *Milch Zentr.*, 1912, 41, 221.

or not used at all. Under these circumstances, according to Sohngen,[1] certain organisms may become sufficiently numèrous to produce action on the fat, and in contradistinction to plant lipases, these microbic enzymes are distinctly thermostable. These organisms can be largely kept jn abeyance by properly aërating the starter or cream and by the rapid development of acidity under proper conditions.

Oleomargarine.

Margarine (Compare Vol. II, p. 313).—During the last few years the composition of margarine has undergone profound changes. These changes, which are still in progress, have rendered the analysis of modern margarine mixtures one of the most complicated problems with which the analyst can be confronted.

Except in the case of pastry and cooking margarines, the use of animal fats is rapidly ceasing. Their place has been taken by coconut and palm kernel products, which often reach 70% in the fatty mixture. As a certain percentage of butter fat is often present, which may either arise from the milk used in manufacture, or be purposely added to improve flavour, a recasting of the methods of analysis was imperative. A very large number of methods of dealing with these mixtures have been published, but the writers[2] are of the opinion that the original Reichert-Meissl method, with the additions of Polenske and Kirschner, is quite sufficient for the resolution of mixtures containing coconut and palm kernel products in the presence of butter fat and indifferent oils. It is only necessary to carry out the process in a standard manner, when the tables and curves proposed by the writers are applicable. *It must, however, be understood that unless the conditions of experiment are carefully adhered to, the tables, etc., will not apply.*

In order that any mistake may be avoided the method as used by the writers is here given.

5 grm. of the fat and 20 grm. of glycerol are weighed into a 300 c.c. flask, and 2 c.c. of 50% sodium hydroxide added. The flask is heated over a flame with constant shaking till it clears suddenly, the soap is then cooled and 100 c.c. of recently well-boiled distilled water are added, until the soap is dissolved. 0.1 grm. of powdered pumice, sieved through butter muslin, is added, and then 40 c.c. of sulphuric acid solution (20 to 25 c.c. of strong sulphuric acid diluted to 1,000 c.c., and the solution adjusted so that 35 c.c. neutralise 2 c.c. of the sodium hydroxide solution). The flask is at once connected with the condenser, and heated with a small flame till the insoluble acids are completely melted; the flame is then increased, and 110 c.c. distilled in 19 to 21 minutes. The temperature of the condenser water should be from 18° to 20° and the dimensions of the apparatus should be the same as given by Polenske

[1] Abs. *Cent. f. Bakt.*, Abt. II, 1912, 35, 331.
[2] *Analyst*, 1911, 36, 333 and with Richmond, *ibid*, 1912, 37, 183.
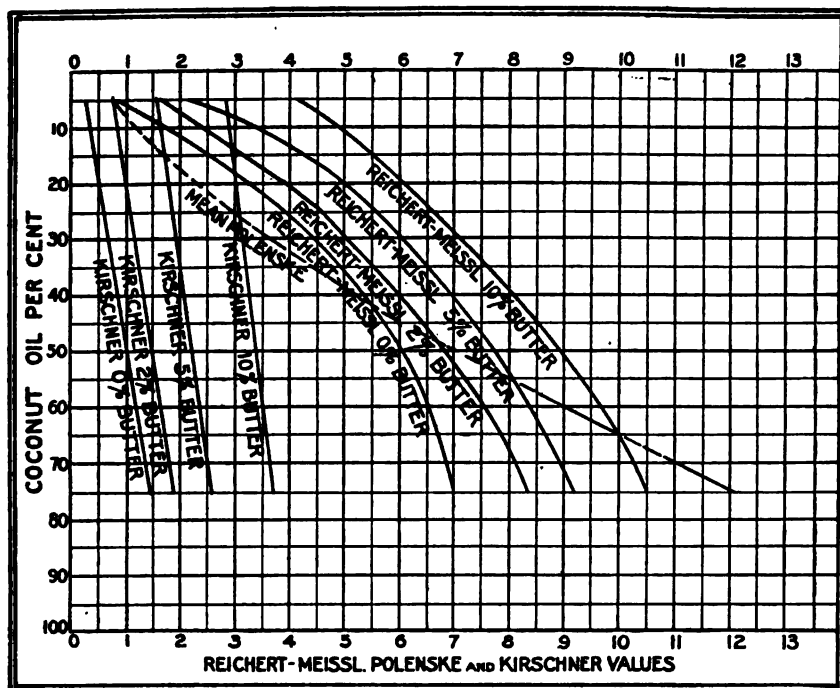
FIG. 8.
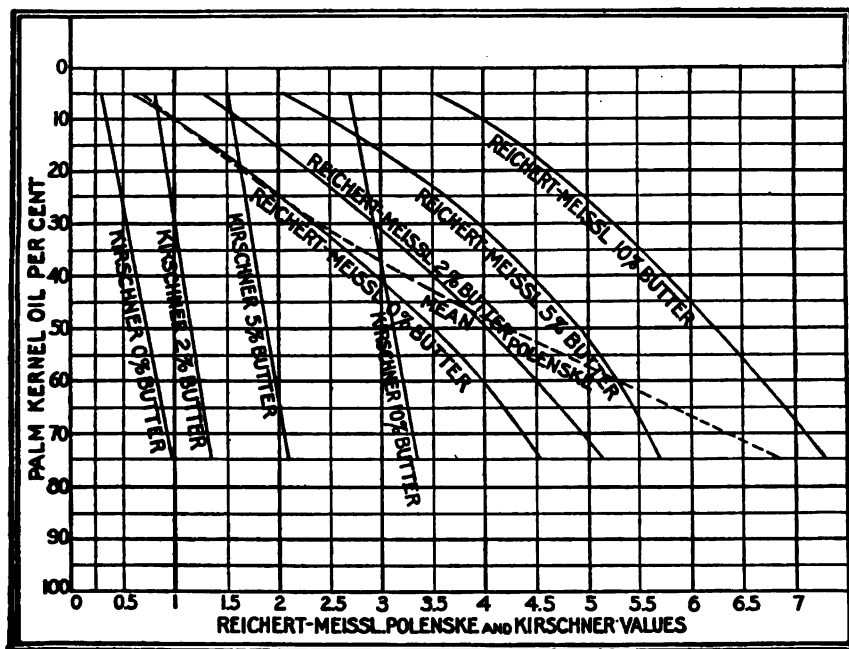
FIG. 9.

(see Vol. II, p. 295). When 110 c.c. have distilled, the flame is removed, and a 25 c.c. cylinder placed under the condenser to catch any drops. The 110 c.c. flask with its contents is immersed in water at 10° to 15° for 15 minutes. After mixing the contents of the 110 c.c. flask, they are filtered and 100 c.c. titrated with $N/10$ baryta, using 0.5 c.c. of a 1% solution of phenolphthaleïn as indicator. This number of c.c. increased by $\frac{1}{10}$, after subtraction of the control (which must be determined in an exactly similar way, using all the reagents except the fat), is the Reichert-Meissl value. The condenser, cylinder, and 110 c.c. receiver, are washed with 18 c.c. of cold water, which are then poured over the filter used to filter the distillate, and rejected. The condenser is washed out with 4 successive portions of 10 c.c. of neutralised alcohol, which are received in the cylinder and poured over the filter into the 110 c.c. flask, the mixed alcohol solutions being then titrated with $N/10$ baryta, using phenolphthaleïn as an indicator. A control value is obtained in a similar way. · The number of c.c. of $N/10$ baryta used, less the number used for the control, is the Polenske figure.

Kirschner's Extension.—To the 100 c.c. of the 110 c.c. distilled and titrated with baryta (care having been taken not to exceed the neutral point) is added 0.5 grm. of finely powdered silver sulphate, and the whole allowed to stand for an hour, with occasional shaking. The liquid is then filtered, 100 c.c. measured off, 35 c.c. of water and 10 c.c. of sulphuric acid (as previously employed) added, together with a long piece of aluminium wire, and 110 c.c. again distilled off in the standard Reichert-Polenske apparatus in 20 minutes; 100 c.c. are titrated, and the number of c.c. so obtained, corrected for the blank, is calculated to the Kirschner value by the following formula:

$$K = x \times \frac{121 \times (100+y)}{10,000};$$

where $x =$ the corrected Kirschner titration;
    $y =$ the number of c.c. of baryta used to neutralise 100 c.c. of the Reichert-Meissl distillate.

From a number of experiments made by the writers the following general deductions were made:

(1) That for the Kirschner values for both coconut and palm kernel oils (with or without admixture of butter fat up to 10%), a straight line can be plotted which will represent, with very great closeness, the values experimentally obtained (see curves).

Further, for any percentage of coconut or palm kernel oils the difference in the Kirschner values for "no butter fat" and for any percentage of butter fat (up to 10%) will be proportional within very small limits to the percentage of butter fat; the closeness of the agreement indicates that the relation will also hold for higher percentages of butter fat.

(2) On examining the values obtained for coconut and palm kernel oils, it is seen that the Polenske value is practically independent of the amount of butter fat present, when present up to 10% in the mixture, and is practically dependent on the presence of the coconut or palm kernel oil only. The mean value was therefore calculated from the four values obtained for mixtures corresponding to each different percentage of coconut or palm kernel oil (see Table I), and on plotting these mean values a regular curve was obtained. The following equations have been worked out for these "mean value" curves:

$$x(\text{C.N.O.}) = 12.3 \ (P - 0.45)^{0.747};$$
$$x(\text{P.K.O.}) = 16.72 \ (P - 0.45)^{0.806};$$

where x = the percentage of coconut or palm kernel oil.

(3) The Polenske value acts as an "indicator," so that when a margarine containing coconut or palm kernel oils is examined by the Reichert-Meissl-Polenske-Kirschner process, reference to the mean curve at once determines the percentage of coconut or palm kernel oil apart from other values. The corresponding Kirschner value obtained from the typical curve then determines the presence or absence of butter fat, the Reichert-Meissl value acting as a confirmatory figure, and controlling the small fluctuations which may in the Kirschner value be occasioned by a variation in butyric acid content of different butters.

On account of the similarity of the results obtained for coconut and palm kernel oils, the following formula will give the percentage of butter present with either fat for the Kirschner and Polenske values found experimentally:

$$\text{Butter per cent.} = \frac{K - (0.262P^{0.63} + 0.09)}{0.242};$$

or nearly as exactly by the more simple formula:

$$\text{Butter per cent.} = \frac{K - (0.1P + 0.24)}{0.244}.$$

The following formulæ connect the Kirschner value and percentage of butter fat when neither coconut nor palm kernel oil is present:

$K = 0.236B + 0.33$, or, with a small increase in the probable error, $K = 0.244B + 0.28$, which is practically the formula given above.

The tables here given for coconut and palm kernel oils, with or without the admixture of butter fat, are the typical values obtained from the curves given above, and are a reliable guide provided the method be carried out under the standard conditions laid down, the standard apparatus being also employed (see Vol. II, p. 295).

It is necessary to draw attention to an observation which the writers have made recently. In the standard apparatus that part of the still-head which passes through the cork and into the interior of the distillation flask is pro-

vided with a small hole in the side to prevent the collection of condensed liquid in the still-head. As originally designed by Polenske, this hole had a fixed distance from the stopper of the flask. Insufficient attention is paid to this point by makers of the apparatus, and the writers have found that if the hole is much more than 1 cm. from the lower surface of the cork, low Polenske values may be obtained with high percentages of coconut oil; and that if this method be made a standard one, particular attention should be given to this point.

TABLE I.

Coconut oil, %	Polenske indicator value		Butter fat			
			0 %	2 %	5 %	10 %
0	0.45	Kirschner................. Reichert-Meissl.............	0.18 0.38	0.80 0.92	1.49 1.70	2.70 3.25
5	0.76	Kirschner................. Reichert-Meisl.............	0.25 0.87	0.75 1.65	1.55 2.15	2.82 4.10
10	1.22	Kirschner................. Reichert-Meissl.............	0.34 1.60	0.84 2.45	1.60 3.42	2.90 4.90
15	1.75	Kirschner................. Reichert-Meissl.............	0.42 2.52	0.92 3.15	1.68 4.35	2.96 5.55
25	2.91	Kirschner.........:......... Reichert-Meissl.............	0.60 3.92	1.08 4.57	1.82 5.55	3.08 6.55
50	7.10	Kirschner................. Reichert-Meisl.............	1.02 6.05	1.50 6.88	2.20 7.72	3.38 8.95
75	12.19	Kirschner................. Reichert-Meissl.............	1.45 7.00	1.92 8.35	2.55 9.20	3.70 10.50
100	16.5	Kirschner................. Reichert-Meisl.............	1.88 8.08	....	....	....

TABLE II.

Palm-kernel oil, %	Polenske indicator value		Butter fat			
			0 %	2 %	5 %	10 %
0	0.45	Kirschner................. Reichert-Meissl.............	0.18 0.38	0.80 0.92	1.49 1.70	2.70 3.25
5	0.68	Kirschner................. Reichert-Meissl.............	0.30 0.53	0.80 1.27	1.51 2.05	2.70 3.50
10	1.00	Kirschner................. Reichert-Meissl.............	0.35 1.00	0.85 1.62	1.54 2.50	2.75 4.00
15	1.35	Kirschner................. Reichert-Meissl.............	0.40 1.35	0.90 2.00	1.57 2.90	2.80 4.35
25	1.97	Kirschner................. Reichert-Meissl.............	0.48 1.97	0.97 2.60	1.65 3.62	2.87 4.97
50	4.22	Kirschner................. Reichert-Meissl.............	0.72 3.50	1.16 4.05	1.87 4.92	3.12 6.22
75	6.87	Kirschner................. Reichert-Meissl.............	0.97 4.55	1.35 5.15	2.07 5.70	3.35 7.30
100	9.82	Kirschner................. Reichert-Meissl.............	1.07 5.22	....	....	....

These methods, while quite satisfactory so long as only coconut *or* palm-kernel oil is present, together with indifferent fats, leave a certain amount to the imagination if both coconut and palm-kernel oils are present together. The resolution of the mixture is then only possible when the percentage of the other fats or oils present is known, which is seldom the case, and it may not be possible to infer the quantity within 10 to 15%. Calculations based on the saponification values are often satisfactory, as by far the larger number of oils which are likely to be used with coconut and palm-kernel products have a saponification value in the neighbourhood of 192 to 195, and the figures for coconut and palm-kernel oils themselves are remarkably constant.

The following addition to the Polenske determination has been made by Burnett and Revis.[1] It gives information as to the relative percentages of coconut and palm-kernel oils in mixtures and may also on occasion throw light on the actual nature of the product present.

In an ordinary "straight" mixture of coconut and palm-kernel oils, the Polenske figure will determine the proportion with at least as great exactness as any other method. For instance, if the Polenske values are plotted as abscissæ, with percentage composition as ordinates, then a straight line joining the points which represent 100% coconut oil and 100% palm-kernel oil respectively will include the Polenske values for all mixtures of these two. The following process is for mixtures containing other constituents:

The ordinary Reichert-Meissl-Polenske determination is made in the *standard* apparatus and by the *standard* method. The Polenske figure is obtained using $N/10$ *baryta*. The insoluble barium salts are then filtered off on a hardened filter-paper under pressure and the salts washed 3 times with 3 c.c. of 93% alcohol (by vol.), the funnel being kept covered during filtration and washing. The paper after all possible alcohol has been sucked out, is dropped into a wide-mouthed CO_2 flask, 10 times the Polenske value in c.c.'s of 93% alcohol[2] (by vol.) added, and the flask boiled under a reflux condenser till the barium salts are in solution. About 5 c.c. of the hot solution are then poured rapidly into a strong test-tube (6 in. × ½ in.), which is at once closed with a stopper carrying a small bulb thermometer and aluminium wire stirrer. The liquid is rapidly stirred, holding the tube in a good light and the turbidity point noticed. The liquid is then warmed till again clear and the turbidity point again noted. This second temperature is taken as the turbidity temperature. If desired, the tube can be fixed in a wider tube so as to obtain slower cooling.

Working in this manner, coconut oil gives a turbidity temperature of 52.5° and palm-kernel oil of 68.5° and mixtures of these fats give temperatures between these limits proportionate to the percentage composition. The turbidity point is very sharp, is independent of the outside temperature and

[1] *Analyst,* 1913, 38, 255.

[2] The alcohol used in these experiments had a sp. gr. 0.8235 at $\frac{15.5°}{15.5°}$. Alcohol of the right strength may be obtained by placing 7 c.c. of water in a 100 c.c. flask and making up to the mark at 15°5° with Kahlbaum's absolute alcohol.

the barium salts, on which the test depends, are quite insoluble in the cold alcohol used for the Polenske determination. The turbidity points are also quite independent of the amounts of the 2 fats present in the original sample, but determine their relative percentages and so supply the necessary information. The strength of alcohol (93% by volume) must be strictly adhered to if the values here given are to be employed. It is the most satisfactory concentration. Other oils and fats (such as are likely to be present) do not interfere. In certain cases small quantities of insoluble volatile acids distil in the Polenske method, which give barium salts insoluble in boiling 93% alcohol. In such cases a clear solution cannot be obtained. The turbid liquid is therefore poured into a long test-tube, corked and kept upright in a water-bath at 70° to 71° until the solid matter has settled. The clear supernatant liquid is then poured off into the turbidity tube and the temperature of turbidity determined. This process does not affect the results. This *permanent* turbidity, due to barium salts of acids other than those derived from coconut and palm-kernel oils, must be distinguished carefully from that due to palm-kernel "stearine." The barium salts of the insoluble volatile acids of this "stearine" do not dissolve in 10 times the Polenske value in c.c. of 93% alcohol, but the liquid becomes more turbid *immediately* the flask is removed from the water-bath.

So long as mixtures of coconut oil and palm-kernel oil are dealt with, the above method gives good confirmatory evidence of the relative percentages. It has been found that coconut oils of different Polenske values give practically identical turbidity temperatures. It is to be noted that the filtration of the barium salts and their subsequent solution must be carried out *within a few hours of the Polenske titration*, as otherwise the salts become partially insoluble and the results are inaccurate.

Mixtures of the "oleines" and "stearines" are sometimes employed in place of the whole oils, but probably rarely. In these cases, although the turbidity temperatures do not give accurate information, on account of the very variable composition of these products, at the same time they give most useful information as to their presence.

The following table gives the results obtained with some of these products.

Fat	Reichert-Meissl	Polenske	Turbidity temperature, ° C.
Coconut oil	7.5	16.5	52.5
Palm-kernel oil	5.2	9.6	68.5
Palm-kernel "oleine"	7.2	12.1	59.5
Palm-kernel "stearine"		8.2	72.5
Coconut "oleine"			53.0 (calc.)
Coconut "stearine"			63.0 (calc.)
Coconut "oleine," 80 per cent. Palm-kernel "oleine," 20 per cent.	8.24	17.05	54.5
Coconut "stearine," 60 per cent. Palm-kernel "stearine," 40 per cent.	4.43	9.93	67.0

It is interesting to note that.cohune oil, which is analytically identical with coconut oil, gives exactly the same turbidity figures

Hardened Fats.

The resolution of margarine mixtures has, however, been still further complicated by the introduction of "hardened" or "semi-hardened" fats (compare page 122). The process of hydrogenation completely destroys the identity of the original fat or oil, except in the case of the saponification value, and if the process be carried to any great extent the liquid vegetable oils begin to assume the properties of the solid animal fats.

The detection of hardened fats thus has become practically dependent on the detection of traces of the catalyst which is commonly nickel. The most delicate test for nickel is the following due to Atack[1] who uses d-benzil-dioxime as the reagent, which has been found to be much more delicate than diacetyldioxime which had been previously employed. Further, the activity of the former reagent is more circumscribed than of the latter (compare page 124).

50–100 grm. of the carefully filtered fat are ignited in a platinum or silica basin, or else shaken out with a 5% solution of hydrochloric acid (sp. gr. 1.14 to 1.16) and the acid aqueous extract concentrated on a water-bath and gently ignited. The residue in either case is taken up with very dilute hydrochloric acid (and if a platinum basin has been used transferred to porcelain), a drop of nitric acid added and the solution evaporated till *almost* dry; a large excess of ammonia (sp. gr. 0.925) is added and then a 0.2% solution of the reagent in ammoniacal solution. A rose-red colour or precipitate indicates nickel.

The relative proportion of ammonia in the reagent used in the above test has been found by the writers to be of importance, but as the personal equation is a factor, it is best for the investigator to make a few tests with known amounts of nickel in order to determine the most delicate combination.

It may be pointed out that a large number of carefully prepared hardened fats have been washed free of nickel by means of hydrochloric acid; these are therefore not detectable by the above process.

It may also be remembered that the process of hardening, if carried to a certain point, results theoretically in the production of a large proportion of triglycerides containing at least 1 molecule of stearic acid. From the observation of hardened fats there is a likelihood that molecules of considerable complexity are actually formed, but as this point has not yet been sufficiently investigated it will be accepted for the moment that stearic acid is the final product.

The presence of this acid in the triglycerides leads to the result that the

[1] *Analyst*, 1913. 36, 316.

usual microscopical test for stearin, after crystallisation of the fat from ether, may give misleading indications. For instance, the same fat hardened in the same manner may give crystals of different appearance, when crystallised in an identical manner on two different occasions. The crystals as a rule approximate to those of beef fat, but, in general, the crystalline conglomerates tend to radiate in all directions from a common centre, whilst the true beef fat conglomerate has usually the well-known fan-like appearance, though in some instances the crystals of hardened fats are practically identical with those of true beef fat.

For these reasons no infallible rules for guidance can be laid down and it is absolutely necessary for the investigator to familiarise himself with the various appearances by the actual examination of many hardened fats, which will enable him to recognise these fats in many circumstances in a way which no verbal description can impart.

From careful observation of these fats the writers venture to suggest that glycerides of varying complexity arise during the process of hydrogenation and if samples be taken at varying periods these complex glycerides behave like eutectic compounds when tested for the melting point, and they have good evidence which suggests that the melting point of the fat and the degree of hydrogenation if plotted as a curve would not result in a straight line.

Analysis of Margarine.—The writers very tentatively venture to suggest the following considerations as a guide to those who have to analyse margarine mixtures.

The disappearance of coconut and palm-kernel oils from the margarine industry is, in view of their extreme utility, very doubtful, and it is to be assumed that they will constitute the major part of the fatty mixture. Nothing as yet has arisen to invalidate the Reichert-Meissl-Polenske-Kirschner process and its findings may be taken as reliable, the further extension of Burnett and Revis being employed in doubtful cases.

The percentage of coconut and palm-kernel products being obtained, it is easy by calculation to arrive at the saponification value, iodine value, etc., of the remaining base.

This remainder may be all a hardened or a partially hardened fat, or a mixture of hardened fat and some other liquid vegetable oil.

If the various tests of Baudouin, Halphen, etc., give negative results, there is at least a probability that the base is all of a hardened variety. The nickel test is then applied, but the absence of nickel will not disprove the presence of hardened fat, though a positive result is of indicative value.

The microscopical appearance of the crystals (if any) obtained from a 25% solution of the fat in methylated ether (more or less according to the rapidity with which the crystals separate) will also point to the presence or absence of hardened fat.

Beyond the above indications analytical methods at the present moment avail but little, and, in any case, the nature of the hardened fat if diagnosed is distinctly problematical.

It has been assumed in the above outline that animal fats are absent.

Ghee.

Ghee, in the strict sense of the word, is the pure clarified milk-fat of the buffalo, sheep, cow or the goat, but as has been shown by the writers[1] it is nearly always adulterated.

In the table below their figures are given for some 16 samples of ghee obtained from different parts of India.

The preparation of ghee is carried out in the following manner: the milk is boiled, immediately after milking, for 1 to 3 hours in earthen pots and when cold is inoculated with some sour milk. When curdled the whole of the milk is churned with a split bamboo for about half an hour, hot water added and the churning continued until the butter "comes." The butter is then skimmed off and kept for a short time when it becomes somewhat rancid.

The butter so produced is heated in an earthen pot until practically all the water present has been boiled away. It is then allowed to clarify and the clear fat, which constitutes ghee, is run into jars while warm.

The fats and oils used as adulterants are very numerous and comprise the carcass fat of various animals together with coconut, ground-nut, cottonseed, poppy-seed, sesame, safflower and niger-seed oils. Of the vegetable fats the most popular adulterant is obtained from the seeds of *B. butyraceæ*, a fat which is very like shea-nut oil in appearance and consistency but fortunately yields very different analytical figures (*vide* page 147). On account of this frequent substitution the fat of *B. butyraceæ* has come in some text-books to be referred to as "ghee."

Kesava-Menon[2] publishes a Reichert-Meissl value of 18.24 for 1 sample of ghee made from buffalo milk. This figure must be regarded as most abnormal and differs entirely from figures of pure ghee published by various observers.

[1] *Analyst,* 1910, 35, 343; 1911, 36, 392.
[2] *J. Soc. Chem. Ind.,* 1910, 29, 1428.

ANALYSES OF GHEE.

Source: Price per cwt.	Rangoon 73s.	Rangoon 71s.	Rangoon 57s.	Rangoon 53s.	Rangoon[1] 40s.	?	Ambala	Ambala	Ceylon
Reichert-Meissl value	30.58	30.42	18.04	11.88	0.44	23.34	29.3	31.5	21.9
Polenske value	1.62	2.42	1.33	0.60	0.47	1.45	2.6	1.66	1.32
Saponification value	228.8	228.7	213.9	206.7	193.4	219.1	223.8	220.1	215.9
Iodine value (Wijs)	30.63	30.75	40.85	45.65	55.73	36.25	30.92	29.60	47.00
Refractometer index at 40° C. (Zeiss scale)	41.4	41.4	44.6	46.3	49.3	42.9	42.3	41.5	45.4
Valenta No. (°C.)	24.0	24.5	47.5	63.0	95.0	....	33.25	23.5	38.25
Sp. gr. 99°/15°C.	0.8632	0.8637	0.8612	0.8603	0.8577	0.8609	0.8624	0.8631	0.8625
Baryta value:[2] (a) Total	312.8	312.6	292.4	282.5	264.4	299.5	305.8	313.5	294.0
(b) Insoluble	251.7	253.3	255.7	257.4	263.3	282.5	254.9	248.7	255.0
(c) Soluble	61.1	59.3	36.7	25.1	1.1	17.0	50.9	64.8	39.0
b − (200 + c)	−9.4	−6.0	+19.0	+32.3	+62.2	+65.5	+4.0	−16.1	+16.0
Free fatty acids (as oleic), %	3.68	2.60	1.80	1.44	0.59	....	1.52	2.59	2.56
Solidifying-point, °C.	....	24.5	28.0	....	34.0	31.0	....	....	....
Unsaponifiable matter, %	....	....	....	....	0.43	....	....	....	....
Inferences	Pure	Pure	Adulterated	Adulterated	Milk fat entirely absent	Adulterated	Slightly adulterated	Pure	Adulterated

[1] Marked "Mixed with grease."   [2] Avé Lallemant's process, see Vol. II, p. 288.

Source	Malabar coast	Bombay City					
		A	B	C	D	B	P
Reichert-Meissl value	24.4	19.00	25.70	26.20	28.40	29.80	25.60
Polenske value	1.8	0.90	0.90	1.50	1.60	1.40	1.20
Saponification value	228.2	219.90	213.80	223.60	224.90	225.40	221.40
Iodine value (Wijs)	....	32.37	37.28	29.24	28.11	29.55	31.83
Refractometer index at 40° C. (Zeiss scale)	42.7	43.10	44.30	44.80	42.00	42.00	42.60
Valenta number (°C.)	....	48.50	43.50	42.50	36.00	33.50	50.00
Sp. gr. 99°/15°C.	....	0.8635	0.8627	0.8619	0.8656	0.8656	0.8618
Baryta values (Avé Lallemant): (a) Total	312.0	300.50	292.30	305.60	307.50	308.10	302.70
(b) Insoluble	256.7	252.20	256.80	256.20	252.40	251.30	256.40
(c) Soluble	55.3	48.30	35.50	49.40	55.10	56.60	46.30
b − (200 + c)	+1.4	+3.90	+21.30	+6.80	−2.70	−5.10	+10.10
Free fatty acids (as oleic)	....	0.37%	2.23%	2.39%	1.83%	1.84%	0.92%
M.p. (°C.)	....	42.50	45.00	43.00	43.00	41.00	43.70
Inferences	Doubtful purity	Adulterated	Highly adulterated	Adulterated	Pure	Pure	Adulterated

ERRATA IN VOL. II.

Page 290, in column 5 of the first table the Zeiss butyro-refractometer numbers given are at 45°, not at 40°, as stated.   At 40° the numbers should read:

44.7
44.2
44.2
44.0
43.7
43.3
42.8
42.8
42.6
42.4
42.1

The last figure in the last column of the end table which is given +6.6 should read +6.0

LARD.

By C. AINSWORTH MITCHELL.

DETECTION OF BEEF AND MUTTON FATS IN LARD.

Bömer[1] has based a method upon the fact that the difference between the melting point of α-palmitodistearin (the characteristic glyceride of lard) and of its separated fatty acids is 5.2°, whereas, in the case of β-palmitodistearin of beef and mutton fats the difference is only 0.1°.

50 grm. of the melted lard are dissolved in 50 c.c. of ether and the solution allowed to stand at about 15°. The crystals are pressed between filter paper, recrystallised from 50 c.c. of ether and again pressed. Should the melting point be less than 61° the crystallisation must be repeated until that melting point is reached. The fatty acids are separated from part of the crystals and the melting point of the glycerides and fatty acids simultaneously determined.

Lards with glycerides melting between 61° and 65° C. must be regarded as adulterated if the melting point of the glycerides added to twice the difference between the melting points of the glycerides and their fatty acids gives a result lower than 71° C.

In the case of glycerides melting between 60° and 61° the presence of beef or mutton fat or of "hardened" oils, q.v., is certain when the difference between the melting point is less than 5° and with glycerides melting from 65° to 68.5° when the difference is less than 3°.

For the detection of hardened oils the method may be used in conjunction with the phytosteryl acetate test.

By means of this test Bömer claims to be able to detect 5% of beef fat in lard containing coconut, arachis or cottonseed oils. Abnormal results due to the presence of fats such as shea butter could be checked by the phytosteryl acetate test (q.v.).

Hydrogenated arachis and sesame oils gave glycerides of high melting point (70.6° to 71.5°), while the corresponding fatty acids melted at 68.6 and 68.5°; hence these glycerides apparently consisted of tristearin.

Hydrogenated cottonseed oil, however, gave a mixture of glycerides melting at 61.3° and containing fatty acids melting at 38°.

The melting point differences ranged from 0° to 0.8° for hardened arachis and sesame oils and reached 2.8° in the case of the cottonseed oil.

[1] Zeit. Untersuch. Nahr. und Genussm., 1913, 26, 559; 1914, 27, 153.

These hardened fats lowered the difference in the melting point of lard to a greater extent than beef fat, from which, however, they could be distinguished by the phytosteryl acetate test.

The least soluble glycerides of the fat of sucking pigs and of pigs fed abnormally upon coconut, maize, sesame and cottonseed-oil cakes differed from those of normal lard in containing a smaller proportion of α-palmitodistearin and more stearo-dipalmitin; but such abnormal feeding did not interfere with the detection of beef fat by this method.

A modification of Bömer's method will detect the presence of a small proportion of lard in coconut oil.

Sprinkmeyer and Diedrichs[1] find that the method will usually detect 5% of beef or mutton fat in lard. In the case of lards rendered in the laboratory the difference between the melting point of the least soluble glycerides and their fatty acids ranged from 4.4° to 7.4°, while Bömer's value (melting. point of glycerides plus twice the difference between the melting point) varied from 73.1 to 76.5.

With beef and mutton fats the difference was 0.8° to 1.2° and the Bömer figure 65.2 to 67.3. Lard containing 5 to 10% of either foreign fat always gave a value below 72 and frequently below 70.

Hydrogenated oils depressed the Bömer value of the lard to the same extent as beef or mutton fat.

[1] *Zeit. Untersuch. Nahr. Genussm.*, 1914, 27, 571.

LINSEED OIL.

By C. A. KLEIN.

Since Vol. II was written, linseed oil has been the subject of extended investigation and during the period covered by the present supplement, over 100 investigations have been published. These investigations have for the most part been directed towards perfecting methods of analysis and obtaining sound data as to natural or artificial variation in the characteristics of the oil. The years 1910–1914 have afforded excellent opportunities for investigation, because linseed oil varied from its normal price to nearly 100% above that figure.

In the following supplement in order to facilitate reference the same order of headings is maintained as that adopted in the original section.

Cultivation.—According to Eyre[1] at the present time there is a growing desire on the part of English agriculturists to return to the cultivation of flax and hemp. Especially is this manifest in Yorkshire, Somerset, Kent, and the East Central Counties, where at one time the cultivation of hemp and line—flax grown for fibre—figured largely in agricultural practice. A general account of the manner of raising good flax crops, and of their subsequent treatment, has been published by Eyre[2] wherein the possibility of reviving the flax industry in England is considered in the light of a report presented to the Development Commissioners in 1912.

Davidson[3] describes the results of a trial growing of flax at Wye (Kent, England).

Bolley[4] has investigated the supposed impoverishment of land devoted to flax culture, which in the past has given rise to the theory that the plant has an exhausting effect on soil, necessitating a change of source of supply.

The experiments of Bolley, carried out at the North Dakota Experimental Station disprove the theory of impoverishment, and establish the fact that flax "wilt" or flax-sick soil is caused by a fungus "*fusarium lini*," and that the fungus is usually introduced with the seed; simple treatment of the seed with formaldehyde will prevent the trouble.

Preparation of Oil from Seed.—Ennis[5] describes in detail modern methods, particularly those adopted in the U. S. A.; this work should be consulted

[1] *Chemical World*, 1913, 2, 310.
[2] *Science Progress*, 1913, 28.
[3] *J. S. Eastern Agric. College*, Wye, 1912, 21, 44.
[4] *Farmers' Bulletin*, 274 of U. S. A. Dept. of Agriculture.
[5] *Linseed Oil and Other Seed Oils*, D. Van Nostrand & Co., New York, 1909.

for technical points. From the standpoint of analysis and control it is germane to point out here that, according to Ennis, 85–90% of the linseed oil produced in the U. S. A. is obtained by crushing. The new process or "percolator" oil has not a high reputation, whilst the linseed meal produced by this process has a low content of oil, as is indicated in the analysis:

Oil	1.5 %
Water	9.18%
Ash	4.90%
Fibre	9.04%
Albuminoids	41.6 %
Carbohydrates	33.78%

Thorpe[1] quotes the following analysis of linseed cake by Kellner:

	Water	Protein	Fat	N-free extract	Crude fibre	Ash
Expressed	11.0	8.6	8.6	31.7	8.7	6.5
Extracted	10.2	37.4	3.8	32.7	9.1	6.8

See Millian[2] for method of detecting carbon disulphide in oil.

Examination of Seeds.—Sheppard[3] gives the following data obtained by the examination of linseed from different localities. The oil was obtained by pressing both the picked and original seed:

	Oil, %	Sp. gr., 15°	Av. weight per seed, mg.	Oleaginous impurities, %	Non-oleaginous impurities, %	Oil in total impurities, %
1. American	39.67	1.1413	4.61	1.5	1.69	10.1
2. American	39.40	....	4.53	1.01	1.05	....
3. La Plata	36.98	1.1415	5.56	0.58	5.64	14.1
4. Calcutta	40.82	1.1326	5.41	4.85	5.03	14.9
5. Bombay	41.23	1.1182	7.88	0.81	2.80	....
6. S. Russia (Keitch)	39.11	1.1375	5.74	5.05	1.71	....
7. N. Russia	36.95	1.1458	4.19	3.31	1.97	....

The oil content was determined by extraction. The average of 11 more recent samples of Calcutta seed was 6.90% of impurities, containing 15.1% oil. The average oil content of the cleaned seed was 41.01%.

Sheppard has made analyses of the oils expressed from the seed when new and after 2 years, and from his results concludes:

(1) Oil pressed from clean linseed does not differ materially from commercially pure linseed oil.

(2) The dark colour of La Plata oil is due to non-oleaginous impurities.

(3) A high percentage of oleaginous impurities does not affect the colour appreciably, but does affect the iodine value slightly.

(4) The technical manufacture of oil by the extraction process does not lower the iodine value.

(5) The constants of the oil pressed from the seed which had been kept 2½ years in a closed container do not appear to be affected by the ageing of the seed.

[1] *Dictionary of Applied Chem.*, Longman's, London, 1912, 3, 323.
[2] *Ann. Chem. Anal.*, 1912, 17, 1.
[3] *Journ. Ind. and Eng. Chem.*, 1912, 4, 14.

The following figures are of interest, representing oil extracted from linseed grown at Enfield Highway, Middlesex, England. The seed was sown in May, 1911, and the plants reaped in October. Owing to continued drought, the plants only attained an average height of 1 ft. and flowered very early. The seed was obviously low in oil content, as only 24.4% of oil could be obtained by extraction methods. The oil, extracted by ether from picked seeds was somewhat green in colour and had the following characteristics:

Sp. gr.................................... 0.931 at 15° C.
Acid No.................................. 3.3
Saponification No......................... 191.9
Iodine No................................. 177.4
M. P. of hexabromides from fatty acids........ 176.0° C.

Neville[1] states that the amount of mucilage in linseed is about 7%.

Linseed Cake.—In continuation of the work of Dunstan, Henry and Auld (Vol. II, p. 326) Auld[2] has found that the bulk of the samples examined yielded a proportion of the "available" hydrocyanic acid by simply moistening with water. The presence of unaltered enzyme thus demonstrated was ascribed to the increased adoption of oil presses of the Anderson type and the expression of the oil in the cold. The amount of hydrocyanic acid was in some cases only very small, and therefore negligible, but in others it was comparatively high and quite sufficient to be distinctly injurious. The amount found in the large number of linseed and mixed cakes examined during the present investigation varied from 0.001 to 0.056%, the latter amounting to 3.9 grains of HCN per pound, being produced by soaking the cake in water at a temperature of 38° C. for 6 hours. In this case, the material smelt strongly of hydrocyanic acid, and when fed to sheep either was not taken readily or made them very sick. The poisonous dose of such a cake is calculated to be one-third of a pound for a sheep and 2 pounds for a calf.

The methods of estimating the hydrocyanic acid are detailed in the original paper, the "total" hydrocyanic acid being obtained by distillation with 6% sulphuric acid and the "free" hydrocyanic acid by soaking in water at 38° C. and subsequently distilling.

The "total" hydrocyanic acid obtained in this way varied from 0.022 to 0.056%, and the free hydrocyanic acid from 0.009 to 0.052%.

Tables are given showing the velocity of formation at 38.5°; it is a function of the time, and is affected by feeding stuffs other than linseed cake. Brewers' yeast appears to contain a cyanogenetic enzyme as it increases the production of hydrocyanic acid; green fodders without exception had an inhibiting effect, as also had glucose and molasses, but cane sugar was without action. Pure cellulose had an inhibiting effect, the cause being proved by experiment and on mathematical grounds to be due to the

[1] Rep. Brit. Ass., 1911, 600.
[2] J. S. E. Agric. College, Wye, 1911, 20, 289.

formation of an adsorption compound of the cellulose and the enzyme, so that the latter was withdrawn from action.

Salts generally retard the action, as also do very weak acids (e.g., 1% HCl) and bicarbonate of soda, which were tried in connection with the effect of the digestive juices.

Experiments on animals proved that the cyanogenetic glucosides are not poisonous when taken in absence of the specific enzymes.

In conclusion the fact stated by Smetham may be emphasised, that if linseed meal is mixed with water much below boiling and then allowed to stand, there is great danger; in order to obviate this it is necessary when preparing a gruel to use boiling water, and to keep the mixture at a temperature of 80° C. or over for at least 40 minutes, under which conditions little or no hydrocyanic acid is likely to be liberated.

The rate of evolution of hydrocyanic acid under digestive conditions has been determined by Collins.[1] The amount of hydrocyanic acid yielded by linseed, and the rate at which it is formed, depend on the quantity of cyanogetic glucosides, on the proportion of enzymes, on the temperature and on the degree of acidity. Normal digestive conditions were obtained as far as possible, and it is shown that, since the acidity of the stomach contents and also that of green grass is approximately $N/20$, linseed cannot under normal conditions produce hydrocyanic acid when fed to carnivorous or herbivorous animals, but abnormal conditions causing reduction of acidity would result in the liberation of hydrocyanic acid. For details the original paper should be consulted, or the undermentioned abstracts.[2,3]

Armstrong and Eyre[4] have applied the Guignard picrate paper test to the detection of hydrocyanic acid produced from the glucoside linamarin, and have found the method to be of extreme delicacy, as hydrocyanic acid can be so detected in a single flax seed. These investigators find that ripe seeds are free from cyanide, but that unripe seeds invariably contain it.

As the flax plant flowers over a considerable period, the harvested seed invariably contains a quantity of unripe seed, and to this cause the presence of linamarin in commercial linseed cake is attributed. The presence of cyanide in linseed cake is usually considered harmful, but the authors consider that instead of this being the case it might be that the peculiar value of linseed cake as a cattle food is determined to some extent by the liberation of small quantities of such a product. All species of *Linum* resembling ordinary flax in habit of growth, carrying white, blue or red flowers, contain more or less hydrocyanic acid, but in no case could this be detected in the yellow flowering species. The amount is subject to variation throughout the period of growth. The enzymic activity of *Linaceæ* is correlated with the presence of the gluco-

[1] *Proc. Durham Phil. Soc.*, 1912, 4, 99.
[2] *J. Soc. Chem. Ind.*, 1912, 31, 507.
[3] *Analyst*, 1912, 37, 313.
[4] *Proc. Royal Soc.*, 1912, 85, B, 370.

side. The yellow flowering species have practically no action on glucosides and incidentally it must be recorded that these species are all slow in germinating and growth. The species containing cyanide are able to hydrolyse linamarin, prunasin and salicin: prunase accompanies linase in both *Phaseolus Lunatus* and in many *Linaceæ*, and as yet linase has not been found without prunase.

Refining.—No change of analytical importance can be recorded here.

Technical Applications.—The high prices which ruled during the early part of the period now covered, had an adverse influence on any new technical applications of the oil. Indeed many users were led to enquire into the technical properties of other drying oils with the result that in certain industries linseed oil has been replaced by the newer oils. This particularly applies to soja bean oil, menhaden oil, and china wood oil, which have replaced linseed oil to an appreciable extent—a change which has been rendered possible by the use of new drying agents. In this connection the papers of Gardner,[1] and Toch[2] should be consulted. China wood oil has been used most extensively as a substitute or rather as an improvement on linseed oil in varnishes. A varnish made from wood oil and rosin is stated to yield more satisfactory results than one prepared from the best Baltic linseed oil and hard gum resins. Mixtures of china wood oil with soja bean and menhaden oil have also been employed successfully in paints. Soja bean oil in white lead paint is considered by Zerr[3] to be preferable to linseed oil, which is of interest in view of the agreement of the German White Lead Cartell and Varnish Makers Assocn. that only linseed or poppy oil should be used.

Chemical Composition.—Haller[4] describes experiments on the "alcoholysis" of commercial samples of linseed oil. The oils investigated had iodine values ranging from 168–176, and saponification values from 185–191. Owing to the insolubility of linseed oil in methyl and ethyl alcohol, esterification was carried out, using benzene, carbon tetrachloride, acetone or ether as solvent. The mixture was boiled with alcohol containing 2.5% of hydrochloric acid. The esters so obtained were fractionated and identified. The methyl esters of palmitic, stearic, oleic, linoleic, linolenic, isolinolenic, and arachidic acid were obtained. Haller states that stearic acid was found in appreciable quantity, whilst the arachidic acid was present in only small quantity.

Erdmann and Bedford,[5] Erdmann, Bedford and Raspe[6] in continuation of previous studies, conclude that the linolenic acid of linseed oil exists in two stereoisomeric forms, which they designate α and β respectively. The α modification on bromination yields the insoluble hexabromide, whilst the β

[1] *J. Frank Inst.*, 1911, 55.
[2] *Proc. Paint and Varnish Soc.*, London, 1910.
[3] *Oil and Col. Trades J.*, 1914, 46, 835.
[4] *Compt. rend.*, 1908, 146, 259.
[5] *Ber.*, 1909, 42, 1324
[6] *Ber.*, 1909, 42, 1334.

variety yields the fluid tetrabromide. It is considered that the chemical composition of linolenic acid is expressed by the formula

$$CH_3\cdot CH_2\cdot CH : CH\cdot CH_2\cdot CH : CH\cdot CH_2\cdot CH : CH\cdot CH_2\cdot CH_2COOH$$

Rollett[1] does not agree with these conclusions and considers that linolenic acid reduced from the hexabromides is an individual acid capable of yielding four distinct stereoisomeric addition bromides. For later discussion see Rollett[2] and Erdmann.[3]

Fahrion[4] gives the following percentage composition as representing normal linseed oil.

Unsaponifiable matter	0.6%
Saturated fatty acids	8.6%
Oleic acid	from 15.0–20.1%
Linoleic acid	30.0%
Linolenic acid	38.0%

By treating the fatty acids of linseed oil dissolved in chloroform with an excess of Hübl's iodine solution, and allowing the mixture to stand, Heiduschka and Rheinberger[5] have prepared trichlorotri-iodolinolenic acid, $C_8H_{20}O_2(ClI)_2$, a white crystalline substance, m. p. 95° C., soluble in chloroform and alcohol.

Morell[6] has examined the saturated acids of linseed oil. A yield of lead salts equal to 6% of saturated acids on the oil taken was obtained, and investigation of these salts showed that the composition of the mixed acids may be summarised as follows:

Stearic acid actually separated	51.7%
Stearic acid present in eutectic mixture	12.7
Palmitic acid present in eutectic mixture	20.0
Residual eutectic mixture	8.0
Oleic acid	4.0
	96.4

It is observed that no daturic, myristic or arachidic acids were detected, but that the methods of separation of the saturated acids are very tedious, and new methods are wanted. By careful working, satisfactory results as to stearic acid are obtainable, but for palmitic, arachidic and myristic acids further investigation of their derivatives is necessary.

de Waele[7] elaborates and improves upon the Fachini-Dorta method of separating liquid from solid fatty acids in oils and fats as follows: 10 grm. of the dry fatty acids are dissolved in 90 c.c. of anhydrous acetone and 10 c.c. of $N/1$ potassium hydroxide are added in a thin stream, constantly stirring. The vessel containing the mixture is then immersed in ice-water for 3–4 hours. The precipitated soaps of the solid acids are filtered off under suction and washed with acetone until the filtrate is colourless. The cake

[1] Zeit. Physiol. Chem., 1909, 62, 422.
[2] Zeit. Physiol. Chem., 1911, 70, 404.
[3] Zeit. Physiol. Chem., 1911, 74, 179.
[4] Zeit. angew. Chem., 1910, 23, 1106.
[5] Chem. Zentr., 1911, 2, 772.
[6] J. Soc. Chem. Ind., 1913, 32, 1091.
[7] Analyst, 1914, 39, 389.

of soap is then removed, dissolved in water with the aid of a little alkali, and the acids separated in the usual manner. The liquid acids in the filtrate can be separated by diluting with water, adding ether, and acidifying. The author claims that a higher iodine value for the liquid acids is obtained than that given by Tortelli and Ruggieri's method, and in addition the process gives quantitative results. The following figures are given for the amounts of saturated acids in various oils:

```
Linseed oil, Calcutta seed.......................  9.1% of iodine value 16.4
Linseed oil, Baltic seed.........................  6.6% of iodine value 20.0
Linseed oil, Northwestern.......................  6.0% of iodine value ....
Soja bean oil...................................  13.8% of iodine value 12.1
Para rubber seed oil............................  16.3% of iodine value 10.7
China wood oil (varies considerably)............  54.1% of iodine value ....
Crude menhaden oil.............................  23.5% of iodine value 20.2
```

Preparation of Sample for Analysis.—The Sub-Committee E of Committee D1 on Linseed Oil appointed by the American Society for Testing Materials (hereinafter referred to as A. S. T. M.) recommends:

"All tests to be made on oil which has been filtered at a temperature of between 60° and 80° F. through paper in the laboratory immediately before weighing out. The sample should be thoroughly agitated before the removal of a portion for filtration or analysis."

Foots.—Walker[1] determines foots by allowing a litre of oil to stand in a clear glass bottle for 8 days, and then noting the amount of sediment found. The highest grades of oil show no turbidity or "foots" by this test. The claim is made that sometimes the separation that could be called foots by the above method is caused by the freezing out of fats of rather high melting point. When a sufficient amount of the sample is available, heat one portion to 100° and set it aside for the determination of foots, together with a sample just as it is received. Note the odour of the warm oil, rubbing it on the hands; a small amount of fish oil may be detected this way.

Break Test.—Heat 50 c.c. of the oil in a beaker to 300° C. Note whether the oil remains unchanged, or "breaks;" that is, shows a jelly-like consistency.

Hertkorn[2] finds that flocculation of linseed oil in boiling is not only caused by carbohydrates or proteins from the seed, but frequently by adulteration with a fatty acid glyceride, which polymerises at 240–260° C. yielding insoluble gelatinous products. China wood oil and candle nut oil are the chief of these adulterants, but certain treated quick-drying train oils behave in the same manner in linseed oil when heated. In this case, however, only "thin" linseed oils could be so adulterated, owing to the greater viscosity of train oils as compared with that of normal linseed oil. It is pointed out that linseed oils containing only a few tenths of a per cent. of china wood oil gelatinise on heating to 240° C. so that adulteration which might not have been intentional would be detected by the test—the suggested unintentional adulteration being the storage of linseed oil in a

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[1] *Bulletin* 109 (revised 1910), *U. S. Dept. Agric. Bureau of Chemistry.*
[2] *Chem. Zeit.*, 1910, 34, 462.

cask which had formerly held china wood oil. The value of Hertkorn's observation is debatable. de Waele states that linseed oil will sometimes show the presence of "Foots" by this test even after 9 months tanking.

Specific Gravity.—Bearse[1] has determined the density and thermal expansion of linseed oil with great precision. From this work it appears that if the density of any sample of pure linseed oil be determined at 25° its density at any other temperature between 10° and 40° may be calculated within the limits of ordinary experimental error by the use of the general equation

$$D_t = D_{25} + a(t-25) + B(t-25)^2$$

in which "a" is taken as -0.0006847 and "B" as $+0.000000120$. Or the density may be measured at any other convenient temperature, and for short temperature intervals the corresponding value of "a" used.

The A. S. T. M. fix

	Max.	Min.
Sp. gr. at $\dfrac{15.5°}{15.5}$	0.936	0.932
or		
Sp. gr. at $\dfrac{25°}{25}$	0.931	0.927

for raw linseed oil from North American seed, and for the determination advise the use of an accurately standardised pyknometer, having a capacity of at least 25 c.c. A test is to be made at 15.5° C. water being taken as 1 at 15.5°, and another test at 25° C. water being taken as 1 at 25°.

Fahrion[2] considers that the above minimum figures are too high. In general, however, any figure below 0.931 demands further enquiry.

Ash.—Walker determines ash by burning 20 grm. of oil in a porcelain dish at as low a temperature as possible, and states that the best oil should contain only a trace of ash. An amount as large as 0.2% would indicate an adulterated or boiled oil. Ash should be examined for lead, manganese, and calcium.

Voorhees[3] found manganese in the ash of 16 samples of pure linseed oil, detecting this by the ammonium persulphate colouration test.

Boughton[4] has examined 16 samples of raw linseed oil of known purity and found them to contain ash varying in quantity from 0.02 to 0.21%. In every instance the ash contained manganese, the amount of which determined by the bismuthate method ranged from a faint trace to 0.0008% calculated on the original oil.

Free Fatty Acids.—The A. S. T. M. fixes 6.0 as maximum acid number, expressed in mgrm. of KOH per gram of oil. Walker determines the acid number as follows: weigh 10 grm. of oil in a 200 c.c. Erlenmeyer flask, add 50 c.c. of neutral alcohol, connect with a reflux condenser, and heat on a steam

[1] *Proc. Amer. Soc. Test Materials,* 1911, 11, 211.
[2] *Die Chemie der trocknenden Oele,* Springer, Berlin, 1911, 9.
[3] *Proc. Amer. Soc. Test. Matl.,* 1911, 11, 209.
[4] *U. S. Dept. Agric., Bureau of Chem.,* Circular No. 111, 1913 and *J. Ind. and Eng. Chem.,* 1913, 5, 281.

bath for half an hour. Remove from bath, cool, add phenolphthaleïn and titrate the free acid with $N/5$ sodium hydroxide. The acid number varies with the age of the oil, and should be less than 8, though when the oil has been refined with sulphuric acid it may show a higher number: test for sulphuric acid.

Unsaponifiable Matter.—The A. S. T. M. fixes 1.5% as limit, and recommends the method of Boemer:[1] To 100 grm. of oil in a 1,000 to 1,500 c.c. Erlenmeyer flask add 60 c.c. of an aqueous solution of potassium hydroxide (200 grm. KOH dissolved in water and made up to 300 c.c.) and 140 c.c. of 95% alcohol. Connect with a reflux condenser and heat on the water-bath, shaking at first until the liquid becomes clear. Then heat for 1 hour with occasional shaking. Transfer while yet warm to a 2,000 c.c. separating funnel to which some water has been added, wash out the Erlenmeyer with water using in all 600 c.c. Cool, add 800 c.c. of ether and shake vigorously 1 minute. In a few minutes the ether solution separates perfectly clear. Draw off the soap and filter the ether (to remove last traces of soap) into a large Erlenmeyer flask and distill off the ether, adding if necessary 1 or 2 pieces of pumice stone. Shake the soap solution 3 times with 400 c.c. of ether, adding the extract to the first ether extract. To the residue left after distilling the ether add 3 c.c. of the solution of potash, and 7 c.c. of the 95% alcohol, and heat under reflux condenser for 10 minutes on the water-bath. Transfer to a small separating funnel, using 20 to 30 c.c. of water, and after cooling shake out with 2 portions of 100 c.c. of ether; wash the ether 3 times with 10 c.c. of water. After drawing off the last of the water, filter the ethereal solution so as to remove the last drops of water, distil off the ether, dry residue in a water oven and weigh.

Iodine Value.—The A. S. T. M. fixes 178 as the minimum Hanus value. Walker gives 174–193, and states that Gill has shown that a pure raw oil may give a value as low as 160. In the U. S. A. the Hanus method is largely used, whereas in England the Wijs method is more frequently adopted. In this connection Smith and Tuttle[2] have investigated the Hanus method, and found that concordant figures were obtained for raw linseed oil when the quantity of oil taken for a determination did not exceed 0.25 grm. When, however, a greater quantity of oil was used the iodine value was decreased. With burnt oils it was found that the limit of agreement diminished with an increased degree of burning. Variation of temperature was found to have more influence on the values obtained for burnt oils than was the case with raw or boiled oils.

Ingle[3] gives the following limits for samples of established purity:

Baltic oil	190–204 iodine value
Indian oils	180–189 iodine value
La Plata	175–186 iodine value
Black sea	176–182 iodine value
North American	177–188 iodine value
Morocco, Dutch and Turkish oils	185–192 iodine value

[1] Ubbelohde, *Handbuch der Öle u. Fette.*, 261–262.
[2] *U. S. Bureau of Standards*, Technologic paper No. 37; *J. Frank. Inst.*, 1914, **177**, 687.
[3] *J. Soc. Chem. Ind.*, 1911, **30**, 344.

Ingle remarks that in all the foregoing values the exact method of determination is the important factor, and emphasizes the necessity of using pure reagents for this purpose. The method he used was that of Wijs.

It is difficult to fix absolute limits for the iodine value as is shown by the work of Meister[1] and Wolff[2] who have obtained low iodine values, viz., 157–166 from linseed oil of undoubted purity. Such figures are, however, not frequent, and would demand special inquiry. The writer considers that values below 165 should be regarded with some suspicion, and further investigation made.

Wilhelm and Meister[3] find glacial acetic acid is the best solvent for oxidised linseed oil (linoxyn or scrim oil), and advise its use in determining the iodine values of such products.

The extent of oxidation to which the oil has been submitted determines its solubility—highly oxidised oils being only partially soluble, even in hot acid. In spite of these drawbacks, the authors prefer this solvent in the place of chloroform or the solvents usually used. In the case of highly oxidised oils, the end point is not so distinct, as with the ordinary mixture.

de Waele finds that the use of a solvent is unnecessary in the examination of linoxyn, if the sample be comminuted by grinding in a mortar. An hour's soaking in chloroform or carbon tetrachloride previous to the addition of the Wijs (or other) solution will also serve to swell the substance and ensure its thorough interaction with the iodine reagent. Complete solution of oxidised oil or linoxyn is more satisfactorily obtained by the use of hot amyl alcohol or acetate as a solvent during 5–10 hours.

Bromine Values.—Vaubel[4] has devised a method of estimating the "Primary and Secondary Bromine Values of Oils." The results obtained are in close agreement with the iodine values of the oils, but the process does not appear to be of analytical importance at present.

Insoluble Hexabromides.—The test devised by Hehner and Mitchell[5] has received considerable attention, because of its great value in the analytical examination of linseed oil. Certain difficulties attend its use so that varying yields are frequently reported, and efforts have been and are being made to standardise the method of working.

Qualitative.—Eisenschmil and Copthorne[6] have devised a qualitative test for fish oils in vegetable oils, based on the insolubility of the fish oil bromides in chloroform, in distinction to the bromides of linseed oil, which are soluble. The method is described as follows: 100 drops of oil are dissolved in 6 c.c. of a mixture of equal volumes of chloroform and glacial acetic acid. Bromine is added drop by drop until the brown colouration remains. After 10 minutes the test-tubes are placed in a beaker containing boiling water.

[1] *Farb. Zeit.*, 1910, 16, 17.
[2] *Farb. Zeit.*, 1910, 16, 120.
[3] *Chem. Rev. Fett. Ind.*, 1910, 17, 260.
[4] *Zeits. angew. Chem.*, 1910, 23, 2077.
[5] *Analyst*, 1898, 23, 310.
[6] *J. Ind. and Eng. Chem.*, 1910, 2, 28.

Linseed oil and other vegetable oils will clear up completely within a few seconds, whilst fish oils will remain cloudy and give a sandy precipitate at the bottom of the tube within a short time. Fish oils that have been heated to 260° or more for some time will not respond to this test.

Trials made by the authors cited show that it is not possible to use this method for quantitative analysis unless a minimum of fish oil present is all that would be desired. The amount of insoluble bromides in oils of the same kind, but from different sources, varies to a considerable extent.

On account of heating and other oxidising processes to which the oils are subjected before they reach the market no reliable quantitative method will ever be worked out on the lines indicated above. The fact, however, that the precipitate formed can be weighed and is indeed in direct proportion to the amount of fish oil present in a given sample, raises this process above the level of an ordinary qualitative test; therein lies an advantage over other methods now in use. The short time required for the test and its applicability to both raw and boiled linseed oil are other factors of importance.

Quantitative Methods.—Ingle[1] applies the hexabromide test in the following manner: 1 to 1.5 grm. of oil are weighed out in tared beaker, and dissolved in 40 c.c. of ether (sp. gr. 0.720) to which 5 c.c. of glacial acetic acid have been added. Bromine is then run in until an excess is shown by the colour of the solution. The mixture is allowed to stand 6 hours, more bromine being added if necessary. After 6 hours the precipitate is collected on a tared filter paper, washed 4 times with 10 c.c. of ether, and after drying the flask (in which some of the precipitate often remains) together with the filter paper in the water-oven the weight of the hexabromide is obtained. Ingle obtained the following figures for samples of undoubted purity:

Oil	Iodine value	Sp gr. at 15° C.	Hexabromides		M. p. ° C.
			1	2	
Baltic oil...............................	197.0	0.9357	48.1	47.5	140–5
Calcutta oil.............................	185.0	0.9322	39.1	39.3	140–4
Dutch crushed oil.......................	182.5	0.9322	36.9	....	140–5
English crushed oil......................	185.0	0.9332	40.0	....	140–3
Plate oil................................	179.5	0.9315	35.3	33.7	140–5
Menhaden oil............................	182.0	0.9328	61.8	....	decomposes
Tung oil................................	168.0	....	nil	....	....

Ingle points out that the yields of hexabromides obtained by him are greater than those given by Hehner and Mitchell; experiments showed that variation in the time of contact with the bromine ether solution did not influence the yield.

Jensen[2] gives the following data in this connection; for method of preparation of hexabromides see original communication.

[1] *J. Soc. Chem. Ind.*, 1911, 30, 344.
[2] *Pharm. J.*, 1911, 86, 839.

Oil from laboratory-pressed River Plate seed:
  Ether insoluble hexabromoglyceride $\left\{\begin{array}{l} 44.0\,\% \\ 118\text{–}141° \text{ m.p.} \end{array}\right.$
Commercial sample, apparently genuine:
  Ether insoluble hexabromides from fatty acids $\left\{\begin{array}{l} 44.9\,\% \\ 124\text{–}142° \text{ m.p.} \end{array}\right.$
Adulterated sample (small quantity of fish oil):
  Ether insoluble hexabromides from fatty acids $\left\{\begin{array}{l} 47.5\,\% \\ 125\text{–}143° \text{ m.p.} \end{array}\right.$

These figures are not in agreement with those usually obtained.

Sprinkmeyer and Diedrichs[1] using Hehner and Mitchell's original method, obtained 28.9% hexabromides.

Eibner and Muggenthaler[2] prepare the fatty acids with all precautions against oxidation, and then brominate at a temperature of −10° C., subsequently filtering on asbestos. The original communication should be consulted for details of the method which, although lengthy, is probably the most exact yet recorded.

The authors made a large number of determinations on Raw linseed oil of different origin, and obtained yields of hexabromides as follows:

Dutch oils.................................... 51.73%
La Plata oils................................. 51.66%
Indian oils................................... 50.50%
Baltic oils................................... 57.96%

The hexabromide value is not appreciably affected by the refining processes. The following values were obtained on applying the method to other oils:

Poppy oil..................................... 0.0 %
China wood oil................................ 0.0 %
Perilla oil................................... 64.12%
Ocumi oil 60.98%
Rape oil 6.34%
Soja bean oil................................. 7.17%

The authors show how rape oil can be quantitatively estimated in linseed oil. When the presence of rape oil has been proved by the erucic acid test, the approximate proportion of the adulterant can be calculated from the hexabromide value, 10% of rape oil reducing the hexabromide value of linseed oil by about 4.4%. 4% should be deducted from the value of rape oil found to allow for the permissible contamination of linseed oil by Cruciferæ.

Gemmell[3] prefers to brominate the fatty acids; for method and data, see page 119.

The percentage of bromine in the insoluble hexabromides varied from 67% to 68.5% against 70.96% for $C_{17}H_{24}O_2Br_8$.

Cod-liver oil yielded......................... 35.20%
Whale oil..................................... 21.70%
Brown whale oil............................... 25.8 %
Menhaden oil.................................. 51.7 %
Shark liver oil............................... 17.70%
Sperm oil..................................... 1.70%

No definite relationship could be traced between the quantity of hexabromide and iodine value.

[1] *Zeit. Untersuch. Nahr. Genussm,* 1912, 23, 679.
[2] *Farb. Zeit.,* 1912, 18, 131, *et seq.* or see abstract *Chem. Zentr.,* 1913, 1, 567; *Analyst,* 1913; *J. Soc. Chem. Ind.,* 1913, 32, 242; *Proc. Amer. Soc. Test. Mtrls.,* 1913, 13 393.
[3] *Analyst,* 1914, 39, 297.

For Sutcliffe's[1] method, see page 118.

At the present time no particular method can be definitely recommended, though for exact work the method of Eibner and Muggenthaler appears to be the best. In carrying out the test the writer finds that in order to obtain comparative results the following points must be recognised:

(1) For quantitative work some standardised method must be employed, and the yields compared with that given by a sample of known purity, determined under exactly the same conditions, and preferably at the same time.

(2) A low temperature is advisable.

(3) The hexabromides should be prepared from the fatty acids, otherwise, if fish oil be present, difficulty will be experienced in filtration.

(4) The melting point of the hexabromides should be determined. Hexabromides from linseed-oil glycerides melt at 143–147°; those from the fatty acids at 177°. 10% of fish oil can be readily detected, as the insoluble bromides obtained from such a mixture blacken and decompose instead of melting.

Refractive Index.—The A. S. T. M. advises using an Abbé Refractometer at 25° C. and fix 1.4805 and 1.4790 as maximum and minimum respectively.

Klimont[2] has determined the refraction constants of linseed oil according to the method of Procter,[3] and gives the following figures:

Refractive index...................... 1.4705–1.4810
Mean molecular refraction............. 447
Saponification value.................. 193.7

The mean molecular refraction was calculated from the formula

$$(n - 1)\frac{M}{d} \text{ or } \left(\frac{n^2 - 1}{n^2 + 2}\right)\frac{M}{d}$$

where n represents refractive index.

d represents sp. gr. at same temperature.

M represents mean molecular weight calculated from the saponification value.

White and Thomas[4] state that as the refractive index of china wood oil (1.5560 at 25° C.) is much higher than that of linseed oil, the determination of the refractive index might be of value in detecting adulteration. The increased viscosity of china wood oil at 50° may also be of use in this connection.

Oxygen Absorption and Drying Test.—The earlier method of Livache, Bishop and Weger, in which the increase in weight of films during drying was determined, has been modified by various workers.

Powney[5] from a series of experiments undertaken to determine the influence

[1] Analyst, 1914, 39, 28.
[2] Zeit. angew. Chem., 1911, 24, 254.
[3] J. Soc. Chem. Ind., 1898, 24, 254.
[4] J. Ind. Eng. Chem., 1912, 4, 878.
[5] Analyst, 1910, 35, 192.

of turpentine and turpentine substitutes on the drying of linseed oil (boiled and raw) concludes:

(1) Except in the case of raw oil, none of the solvents accelerate the drying to any marked degree; turpentine is on the whole the most suitable.

(2) Suitable turpentine substitutes (such as the mixture of petroleum hydrocarbons and turpentine used in the experiments) may possess such relatively high efficiency as to warrant their employment for many purposes in place of pure turpentine.

A modification of the Livache method is due to Liverseege and Elsdon[1] in which 10 grm. of finely powdered litharge (dried to constancy at 20°–22°) is spread evenly over a flat bottomed dish of German silver 3 in. in diameter by 1 in. deep. The whole is weighed and 0.7 to 0.9 grm. of linseed oil added. After weighing, 5 c.c. methylated ether (0.720 sp. gr.) are added. The moistened litharge is spread evenly over the bottom by gently rocking the dish, which is then put into an incubator for one, two or more days until the weight is constant, or has passed its maximum. The gain in weight is expressed on the oil taken. The ether is almost wholly volatilised in 30 minutes, and tests show that the amount of non-volatile residue left therefrom does not exceed 3 mg., and is often less. The results of the test may be summed up as follows:

(1) Genuine raw linseed oil ceases to gain weight after 2 days, such gain being from about 15% to 18%, depending on the quality of the oil.

(2) Genuine boiled linseed oil ceases to gain after 1 day, the amount being fairly constant, viz., 12% to 14%.

(3) Whilst drying oils do not gain after about 2 days, non-drying oils may continue to do so for weeks.

(4) When oils of a particular class are compared, the gain in weight varies with the iodine value, and there is evidence of some relationship between the figures for oils of different classes.

Wilson and Heaven[2] have devised a method of determining the oxygen absorption of drying oils, which differs from those generally used, in that instead of determining the increase in weight of a drying film, they measure the volume of oxygen absorbed. The method has the merit of being rapid—1 hour's absorption at 100° being sufficient if the oil is distributed over a large surface.

The difference between the various classes of drying, semi-drying, and non-drying oils is clearly brought out by the results obtained. The method ignores, however, the evolution of volatile products during the drying of linseed oil, and thus far is open to criticism.

Krumbhaar[3] has modified the volumetric absorption method in such a way as to provide for the absorption of the volatile products. The oil is oxidised

[1] J. Soc. Chem. Ind., 1912, 31, 207.
[2] J. Soc. Chem. Ind., 1912, 31, 565.
[3] Chem. Rev., 1913, 20, 287.

in a large glass flask containing soda lime as absorbent of acid volatile products, and is connected with a paraffin oil eudiometer. Krumbhaar finds that $9\frac{1}{2}$ hours at 30° C. suffices for the maximum absorption, and by calculation from the diminished pressure the volume of gas absorbed is obtained. An absorption of 41% was obtained, this being in agreement with the early result of Weger, viz., 43.7% (compare page 192).

Mannhardt[1] uses aluminum plates for the drying test. The apparatus consists of 5 such plates, 3 in.×6 in., showing a total surface of 180 sq. in. The plates are held by an aluminum wire frame at intervals of about $\frac{3}{8}$ in. The whole apparatus weighs less than 80 grm. and can be conveniently weighed on an analytical balance. The quantity of oil used is between 0.5 and 0.7 grm. It is usually applied at the rate of about 2 drops per surface, and is uniformly distributed with the tip of the finger. In order to obtain reliable results, observance of the following conditions is essential:

The tests should be carried out:

(a) Along with a control oil of known purity or character.
(b) Using uniform amounts of drier.
(c) Using uniform temperature.
(d) Using a definite limited thickness of film.
(e) In an atmosphere of moderate humidity.
(f) With free access of air.
(g) Under the same degree of illumination.

Elsdon and Hawley[2] describe a method of detecting adulteration of linseed oil by a process involving oxidation for a specified time, followed by subsequent extraction and determination of unoxidised oil. The process is carried out as follows: 5 c.c. of a solution of 2.5 grm. of oil in 25 c.c. of ether are distributed over an Adam's coil of filter paper. After drying overnight, the coil is heated on the shelf of a steam-oven for 2 hours, and then extracted in a Soxhlet extractor for 3 hours, using ethyl ether (0.720) as solvent. The extract is evaporated and a little alcohol added to the residue, after which the extraction flask is heated for 2 hours in a steam oven, and then weighed. Ten samples of linseed oil gave 14.0% to 19.2% extract, colza oil 100.6%, linseed oil plus 20% colza oil, 21.0%; linseed oil plus 20% seal oil, 31.6%. It is suggested that the maximum permissible extract is represented by the equation

$$\text{Extract} = 81.9 - 0.35I$$

where I = iodine value (Wijs) and that routine testing should include the determination of iodine value and quantity of unoxidised extract as above.

In connection with this test it is to be noted that many cases of adulteration by semi-drying oils (excepting those at the bottom of the scale, e.g.,

[1] *J. Ind. Eng. Chem.*, 1913, **5**, 129.
[2] *Analyst*, 1913, **38**, 3.

cotton seed) would have to be in quantities well over 50% before any information would be learned from this method. An exception is to be made in the case of the so-called "drying" fish oils, menhaden, e.g., which yield no ether-insoluble body whatever. This is the best test for a fish oil.

Drying on Glass.—Walker coats glass plates 3 in.×4 in. with the oil to be examined, and exposes to air and light, noting when the film ceases to be tacky. A good oil should dry to an elastic film in 3 days. Varying conditions of light, temperature, and moisture have such an influence on drying tests that for comparison of one linseed oil with others all samples should be examined at the same time.

Davidson[1] has standardised a method of determining the rate of drying of oils on glass. In this method use is made of an incubator giving a constant temperature of 100° F. and a setting value is obtained by ascertaining the time required for a film of oil to become set as determined by its being sufficiently firm to bear the finger being drawn gently across it without leaving a whitish mark. Ten minutes is taken as the unit for the expression of the setting value. Concordant values appear to be obtained by the method which has been applied to a study of the action of driers on linseed oil.

Qualitative Detection of Adulterants in Linseed Oil.—To detect fish oils in linseed oil see "Hexabromide Test" by Eisenschmil and Copthorne (page 189).

Outerbridge, Jr.,[2] has shown that traces of mineral or rosin oil can be detected in linseed oil by the former showing a green and the latter a blue fluorescence when such a mixture is exposed to ultra-violet light (preferably an ordinary enclosed arc light) and examined against a black background. The author claims to have detected 0.1% of mineral oil in a mixture which by other methods appeared to be non-fluorescent. Samples of "debloomed" mineral oil (which by the light of an ordinary arc or in sunlight appeared free from bloom) became highly fluorescent when examined in ultra-violet rays. The test is not applicable to boiled or polymerised oils which in themselves are fluorescent.

Thurston[3] finds that the Liebermann-Storch test does not detect 50% of rosin oil in linseed oil, and that 1 drop of conc. nitric acid added to 5 drops of the oil gives a more reliable indication. Rosin or "gloss oil" gives a red-violet tint, changing to violet red. The same colour is given by 50% linseed-oil-rosin-oil mixture, whilst a yellow to greenish-yellow colour is given after standing a few seconds by mixtures containing 5%–25% of rosin oil.

Walker carries out the Liebermann-Storch test as follows: To 20 c.c. of oil add 50 c.c. alcohol, teat on steam-bath for 15 minutes, cool, decant the alcohol, evaporate to dryness, add 5 c.c. acetic anhydride, warm, cool, draw off the acetic anhydride and add a drop of sulphuric acid, 1.53 sp. gr. Rosin or rosin oil gives a fugitive violet colour.

[1] *Proc. Paint and Varnish Soc.*, London, 1908, 1910.
[2] *Proc. Amer. Soc. for Testing Matrls.*, Philadelphia, 1911, 11, 1.
[3] *Oil, Paint and Drug Reporter*, 1914, 50.

The period of abnormally high prices previously referred to clearly showed that adulteration of linseed oil is not always easily detected. Clumsy adulteration with mineral oils or rosin is of course readily detected by analysis, but the experience of the writer is that such adulteration is rarely practised. The type of adulteration most difficult to detect is the admixture of linseed oil with other seed oils, the characteristics of which do not seriously affect those of linseed oil on admixture in reasonable proportion. The mixture can often only be classed as a low-grade oil and positive evidence was obtained of admixture of this description. The following example is one in which the mixing had been overdone, with the result that the oil, which had been tendered as "Genuine Linseed Oil," was refused, and damages established.

Unsaponifiable matter	1.39 %
Sp. gr. at 15½° C	0.9315
Acid number	6.72
Iodine value	148.5
Saponification value	175.7

The A. S. T. M. state that owing to the fact that it is possible to adulterate raw linseed oil with other vegetable oils to a considerable extent without detection, the sub-committee is working to devise a test of more value than those ordinarily in use. The determination of the hexabromides seems to offer possibilities, and in consequence the Society has decided to investigate this test.

Interpretation of Abnormal Characterisitcs.—The foregoing information refers principally to raw and refined linseed oil under normal conditions, and it now becomes necessary to point out in what manner the characteristics may be altered as a result of legitimate influences to which the oil has been submitted.

Effect of Storage.—Storage in closed vessels does not appear. to effect any considerable change. The following values were obtained by the writer from a sample of genuine Baltic linseed oil, which had been stored in a closed vessel for 40 years:

Sp. gr. 15½° C	0.9348
Saponification value	190.3
Acid number	4.32
Iodine value	166.6
Unsaponifiable matter	0.67 %
Yield of hexabromides	22.4 %
M. p. of fatty acid, hexabromides	177.0° C.

These figures appear to confirm the opinion of Fahrion that during storage the unsaturated acids polymerise, but the effect is not very marked in a period of 40 years.

Hydrolysis of Glycerides.—Gardner[1] is of the opinion that certain changes which take place in oils and paints during storage are to be ascribed to auto-hydrolysis of the glycerides by enzymes or bacterial infection from low-class or imperfectly sterilised seed.

The most frequent effect observed is the increase in the free acid value

[1] *J. Franklin Inst.*, 1914, 5.

of the oil, and when certain pigments, particularly those having a lead base, are ground in such oil, there is a marked tendency to "livering," or the formation of granular lumps in the paint. To overcome the difficulty, it is desirable to heat cloudy linseed oil to 100° and so sterilise it; the cloudiness is considered to be an indication of possible contamination.

Oil Extracted from Paints.—Oil extracted from paints, although genuine, frequently yields abnormal values. The oil is often bleached by contact with the pigment.

Gardner[1] has determined the effect of pigments ground in linseed oil and finds that after 1 month's interaction with the oil, the pigment is readily detected in the case of lead and zinc compounds, as shown by the ash content of the extracted oil. Barytes, silica and other so-called inert pigments showed no change and the author is of opinion that the increased rate of drying observed by Sabin when using such mixtures must be ascribed to the increased surface presented and not to any chemically induced effect of the pigment.

More complete enquiries have been undertaken by Gardner,[2] and Boughton,[3] and the results of these workers are tabulated below.

ORIGINAL OIL.

Pigment used	Sp. gr. G 0.932 / B 0.934			Ash, % G 0.19 / B 0.13			Iodine value G 181.0 / B 179.6			Acid value G 2.5 / B 1.7		
	2 years	1 year	2 years	2 years	1 year	2 years	2 years	1 year	2 years	2 years	1 year	2 years
White — Zinc oxide	0.9237	0.935	0.934	0.360	0.25	0.13	161.0	181.3	179.7	3.5	....	.....
Basic carbonate white lead	0.9372	0.940	0.938	1.149	0.35	0.40	157.5	175.0	177.3	8.6	....	.....
"Leaded zinc"	0.9389	....	.....	0.922	....	.....	157.4	....	.....	5.7	....	.....
Basic carbonate white lead 50%... Zinc oxide 40%... Barytes 10%...	.....	....	.....	0.674	....	.....	154.1	....	.....	6.7	....	.....
Basic sulphate white lead 60%... Zinc oxide 40%...	0.9334	....	.....	0.626	....	.....	157.8	....	.....	5.6	....	.....
Barytes	0.9325	....	.....	0.212	....	.....	169.6	....	.....	3.5	....	.....
Silica	0.9465	....	.....	0.204	....	.....	149.2	....	.....	8.7	....	.....
Kaolin containing CaSO₄	......	0.939	0.936	......	0.12	0.14	.....	173.0	171.6	....	....	.....
Black — Carbon black	0.9356			0.195			163.0			10.5	....	.....
Graphite		0.934	0.933	0.201	0.21	0.15	158.5			13.3	....	.....
Artificial graphite		0.935	0.939			0.15		181.0	180.0		....	.....
Lamp black. (See carbon black).												
Black magnetic oxide	......	0.937	0.935	......	0.17	0.13	.....	174.6	173.2	....	....	.....
Red — Red lead				15.56	....	.....	135.4	.....	......	19.2	....	.....
Iron oxide	0.9457	0.941	0.939	0.456	0.15	0.14	156.3	173.8	172.5	8.6	....	.....
Basic chromate of lead	0.9390			1.271			156.7			8.3	....	.....
Yellow — Zinc chromate	......	0.934	0.934	......	0.20	0.18	.....	180.2	179.5	....	....	.....
Chrome yellow	......	0.937	0.935	......	0.14	0.14	.....	176.3	175.7	....	....	.....
Chromium oxide	......	0.937	0.937	......	0.01	0.05	.....	178.0	180.2	....	....	.....

1 J. Ind. Eng. Chem., 1911, 3, 628.
2 J. Franklin Inst., 1912, 174, 415.
3 J. Ind. Eng. Chem., 1913.

The method adopted by Gardner consisted in grinding various pigments with linseed oil to paints of approximately the same consistency and leaving the products in air-tight vessels for nearly 2 years. The vessels were occasionally agitated for the first part of the period and finally were allowed to remain undisturbed for almost a year before examination. The vehicle was then extracted by benzene and petroleum ether and after removal of the solvent was examined, giving the results as summarised marked "G."

Boughton mixed the pigments with raw linseed oil and kept the mixtures in stoppered jars of uniform shape and size for periods of 1 and 2 years in dim diffused light. At the end of each period the contents of the jars were thoroughly mixed and samples taken. Ether was used for the extraction of the oil. The results are tabulated under "B," those obtained from the 1 and 2 year samples being so marked.

It will be seen that no general conclusions can be drawn from these figures. From a technical point of view the figures of Gardner more nearly represent actual conditions, although even here the information is not capable of general application. In practice the conditions differ considerably from those obtaining in the laboratory mixing of oil with pigments and the final results are not the same. The writer's experience shows that the changes taking place vary considerably, the variation being dependent on methods of manufacture. In practice the temperature of grinding is sometimes sufficient to cause to a small extent hydrolysis or polymerisation, depending on the presence of water or otherwise. The following figures show the variation which occurs:

	Ash, %	Sap. value	Sp. gr.	Iodine value
Oil from white-lead linseed-oil paste 10 years old..............	0.47	....	....	....
Oil from white-lead linseed-oil paste 6 years old..............	0.03	....	0.9534	116.0
Oil from white-lead linseed-oil paste 1 year old..............	....	....	0.9600	149.0
Oil from white-lead linseed-oil paste 3 months old............	....	....	0.9500	142.5
Oil from white-lead soja-bean-oil paste 3 weeks old	....	192.2	0.9410	112.3

In these circumstances no definite conclusion can be drawn and the possibility of interaction between oil and pigment must be recognised in the analysis of linseed oil extracted from paints.

Drying of Linseed Oil.—The problem of the drying of linseed oil has been further investigated by numerous workers, but little information of analytical importance obtained. The present position may be summarised as follows:

The drying of linseed oil is essentially an oxidation process, the final oxidation product being as yet unknown. During the drying process considerable quantities of volatile products are formed, the quantity depending on several factors. The so-called oxygen absorption figure obtained by ascertaining the increase in weight of a film of oil on exposure to air does not

represent the total quantity of oxygen actually taking part in the drying process, as no account is taken of that contained in the volatile products: thus Olsen and Ratner[1] found that after 74 days, drying linseed oil had increased in weight 18.05%, whilst the volatile products collected by potash and calcium chloride amounted to 5.21% and 14.55% respectively—the total oyxgen absorbed (assuming the increase in weight of the KOH and $CaCl_2$ to be CO_2 and water) amounted to 37.80%, the linseed oil having lost 1.87% of its carbon and 14.73% of its hydrogen in the process of drying. Friend[2] after 65 days found an increase in weight of oil of 9.35%, whilst volatile products amounted to 14.94%, giving a total oxygen consumed of 24.29%. The variation in these figures illustrates the variable type of results obtained in investigations of this kind.

Sabin[3] has shown that films of linseed oil or paint containing linseed oil increase in weight to a maximum in less than a week, then these films begin to lose weight, though not so rapidly as they had gained.

With raw linseed oil the decrease in weight after 8 months was about 9/10 of the increase, and even at this stage the decrease was continuing. It therefore appears that the oxidation of linseed oil has no definite end-point, solid linoxyn apparently still losing volatile matters and becoming transformed into the fluid superoxidised linseed oil described by Reid. For analytical purposes the limitations of present experimental methods must be taken into account when considering the results obtained by the various methods proposed. The volatile products evolved during the drying of linseed oil have been shown to have definite germicidal value and the hygienic value of paint has been demonstrated in this connection. These products have been shown to contain the lower fatty acids and aldehydes. There is distinct evidence that the chemical constitution of the oxidised product and the volatile products evolved by drying oils varies with the temperature of oxidation.

Fahrion[4] from a study of the drying processes of linseed oil is of the opinion that the chemistry of the drying of the oil is essentially the same as that of the drying of the fatty acids and further that the addition of siccatives does not alter the process, except in so far as auto-oxidation is accelerated.

Fahrion[5] gives the following characteristics of a linoxyn film obtained by drying a film of linseed oil on glass plate for 10 days:

Fatty acids	25.3%
M. p. of acids	38.0° C.
Iodine value of acids	42.9
Hydroxy acids, soluble in ether	40.2%
Hydroxy acids, soluble in alcohol	20.2%
Iodine value	28.3
Hehner value	85.7

Ingle[6] is of the opinion that he has established the following points:

[1] Eighth Int. Cong. Appl. Chem., 1912, Sect. Ve Orig. Common., 12 165.
[2] Proc. Paint and Varnish Soc., London, 1914, 6, 145.
[3] J. Ind. Eng. Chem., 1911, 3, 2.
[4] Zeit. angew Chem., 1910, 23, 722.
[5] Farb. Zeit., 1912, 17, 2530 et seq.
[6] J. Soc. Chem. Ind., 1913, 32, 639.

(1) In the oxidation of a drying oil in air, the amount of oxygen absorbed in dry air is in the ratio of 2I to 2O, but if the air be moist the peroxides thus formed are decomposed with the production of volatile compounds—aldehydes and acids.

(2) That the free acids of linseed and other oils only absorb half the amount of that absorbed by their glycerides. The same remarks apply to their ethyl salts, these only absorbing 1 atom of oxygen for every 2 atoms of iodine absorbed.

Fritz and Zymandi[1] give the following values for oxidised linseed oil:

	Walton oil, 4 samples	Rapid oxidation oil, 2 samples	Runnings, 1 sample
Consistency	Hard.	Very soft.	Very soft.
Sp. gr. water at 4° C.	1.0862 at 15° C. to 1.0734 at 21° C.	....	1.0693 at 20° C.
Iodine value Wijs	61.8 to 65.5	96.2(1)	98.4
Ash	1.16% to 1.41%	0.15%(1)	4.36%
Unoxidised fatty acids	26.2% to 31.2%	43.5% to 49.4%	50.7%
Oxidised fatty acids	46.4% to 56.4%	42.1% to 42.5%	20.0%
Water soluble fatty acids	5.5% to 8.6%	2.7% to 5.7%	8.3%

Driers.—According to Metz[2] the German Lake and Colour Industry Protection Society have resolved that pure linseed varnish should not contain more than 2% of siccatives, or if resinates be employed, not more than 5%.

The more general adoption of cobalt driers and tung-oil driers is of sufficient importance to receive mention, as these driers are likely to be more frequently met with in the future.

Influence of Light on Drying.—Friend (*loc. cit.*) has investigated the influence of colour of pigments on the drying of paints, by using lakes of varying colours, the "base" of which was barium sulphate so selected because of its chemical inactivity.

Boiled Oil.—Ingle[3] shows the change in characteristics effected by the action of heat on linseed oil in the following table:

	Iodine value	Sp. gr.	Hexabromides
Original oil	179.5	0.9315	35.3
After 2 hours	175.5	0.9350	30.7
After 4 hours	170.0	0.9383	27.4
After 6 hours at 195 to 200°C.	165.0	0.9408	26.2
After 10 hours	154.0	0.9501	16.0
After 15½ hours	145.0	0.9583	10.5
After 43 hours	121.0	0.9800	0.9

The polymerisation effected by boiling is clearly shown in the above figures.

de Waele[4] gives a new and simplified method of estimating rosin in mixtures with oils, with a further application of the same method to determine

[1] *Chem. Rev. Fett. Ind.*, 1914, 21, 43.
[2] *Chem. Zeit.*, 1911, 35, 473.
[3] *J. Soc. Chem. Ind.*, 1911, 30, 344.
[4] *Oil and Col. Trades' J.*, 1914, 46.

the glycerol in the oil. 10–20 grm. of the oil-rosin mixture are dissolved in at least twice its weight of amyl alcohol and about 1% of aqueous hydrochloric acid added. The mixture is kept on a steam bath under a reflux condenser for 2–3 hours after which esterification to amyl esters of the fatty acids will be complete, whilst the rosin acids will remain unesterified. The rosin is separated by neutralising with soda, separating off the aqueous layer and liberating the rosin acids under ether in the usual way, when they may be either weighed directly (using the factor of 1.07) or estimated volumetrically. The glycerol from the fatty oil may be separated and estimated by shaking out the original esterified mixture with water 2 or 3 times, when it will be found in an aqueous layer. It may be estimated by evaporating at 70° to a thick syrup and determining the concentration from the refractive index.

A private communication by the author giving details of tests of the methods was as follows:

COMPOSITION OF MIXTURES FOR TESTS.

Linseed oil (acid value 5.6).....................	25.0 grm.
Pure oleic acid................................	12.5 grm.
Rosin..	12.5 grm.

1. *Rosin determination:*

Weight of mixture taken.......................	5.6595 grm.
Weight of rosin obtained......................	1.3220 grm.
X 1.07.......................................	1.4145 grm.
Theory.......................................	1.4149 grm.

2. *Glycerol estimation:*

Weight of mixture taken.......................	20.4368 grm.
Weight of thick syrup.........................	1.2262
Refractive index of same at 15.5° C...........	1.4571
Pure glycerol................................	1.084
Glycerol, %..................................	5.3
Corresponding to fatty oil....................	50.5%

Blown Oils.—Marcusson[1] has modified his earlier method[2] of distinguishing between blown rape oil and blown cottonseed oil in lubricating oils having a mineral oil base. In the improved method the fatty acids isolated as before are treated with light petroleum, and the soluble salts further examined by converting into the lead salts and determining the solubility of these in warm ether. The lead salts obtained from blown rape oil are almost completely soluble, whilst those from blown cottonseed oil are only partially soluble, the difference being exaggerated on cooling. By adopting this method, qualitative examination only is necessary, as 25% of blown cottonseed oil in blown rape oil can readily be detected.

The residue of insoluble lead salts calculated on the weight of soluble fatty acids taken from which they were prepared is 14–18% in the case of blown cottonseed oil, fish, and bone oils, and 8% in the case of a strongly oxidised linseed oil. This method is of value when combined with that of Sherman and Falk[3] in which the original iodine value of an oxidised oil is calculated from the increase in sp. gr. which takes place on blowing. For semi- or non-drying oils it is sufficient in practice to add 0.8 to the iodine

[1] *Mitt. K. K. Materialprüfungsamt.,* 1911, **29**, 50.
[2] Allen's Organic Analysis, 1911., Vol. II, p. 370.
[3] *J. Amer. Chem. Soc.,* 1906, **27**, 605.

value of the oxidised oil for every increase of o.oo1. in sp. gr. at 15/15° C.
over that of the original oil. The iodine value of the oxidised oil can be
taken as 90% of that of the oxidised acids and the sp. gr. of the original
oil as 0.919 for rape and cottonseed oils. The normal iodine values of rape
and cottonseed oil are fairly close, and therefore the qualitative test of Mar-
cusson is of value in identifying the oil.

Fahrion[1] has further modified the method of estimating rosin in blown
oils, in order to obtain increased accuracy. In this later method, 5 grm.
of the oil (freed from metals) are dissolved in 50 c.c. petroleum ether and the
solution treated with 20 c.c. of 96% alcohol, then neutralised with $N/1$
alkali, using phenolphthaleïn as indicator. Water is then added to effect
a dilution of about 60% alcohol and, after shaking, the mixture is allowed to
stand over night. The soap solution is diluted to at least 200 c.c., acidified
with hydrochloric acid and extracted twice with petroleum ether. The
united extracts are esterified with absolute alcohol. After esterification is
complete, phenolphthaleïn and $N/1$ alkali are added until a red colour is just
obtained. The solution is diluted with water until the alcohol is of 60%
strength and from the alcoholic solution of the rosin soap the rosin acids are
obtained in the usual way. Any traces of soap are removed from the
solution of esters by washing with 10 c.c. of 60% alcohol and the washings
added to the solution of the rosin soap.

It is pointed out that the fatty acids insoluble in petroleum spirit are
more difficult to esterify in the case of oxidised oils than is the case with
unoxidised oils. Thus, a known rosin-free blown oil gave a yield of 0.86
of apparent rosin by this method. Against this is the fact that colophony
contains from 10 to 25% (average 14–15%) of neutral substances and hy-
droxyabietic acids, which escape determination. Fahrion uses the following
corrections to compensate for errors created by the foregoing circumstances:

Amounts below 1% are ignored, those between 1 and 4% are taken directly
as rosin, whilst figures above 4% are multiplied by 1.17, a factor correspond-
ing to the average loss of 14–15% of neutral substances and hydroxyabietic
acid.

According to de Waele (*loc. cit.*) the method already quoted can be used
to estimate solid acids in an oxidised oil by working on the "fatty acids freed
from oxidised acids" obtained in Fahrion's method of separation by petroleum
ether. The author states that "polymerised acids" interfere with the process
as these acids appear as "solid acids" but differ from them in that they have
high iodine values and a different appearance.

See also Wolff and Scholze.[2]

For fuller information, on linseed oil, the work of Fahrion[3] is recommended.

[1] *Chem. Rev. Fett. Harz. Ind.*, 1913, 150 *et seq.*
[2] *Chem. Zeit.*, 1914, 38, 369.
[3] *Die Chemie der trocknenden Öle*, Springer, Berlin, 1911.

ERRATA IN VOL. II.

Page 326, line 21, "Dunston" should read "Dunstan."

Page 329, line 19, "differs" should read "differ."

Page 331, line 12, delete the "p" after "increase."

Page 331, line 2 from bottom, "20.5–22.5" should read "205–225."

Page 342, bottom line, for "linolenic hexabromides" read "hexabromo-glycerides."

Page 343, line 1, after "decomposition" insert "at 143.5 to 144.5°, those prepared from the fatty acids."

Page 343 in table, under "Max.," for "0.836" read "0.936" and for "192" read "195."

Page 360, line 2 from bottom, for "Dunlap" read "Dunlop."

Page 365 line 4 from bottom for "Proctor" read "Procter."

SOAPS.

By J. R. POWELL.

In the following section no attempt is made to reorganise the methods of analysis given in Vol. II, but simply a few suggestions are given under the heads of the principal estimations, so as to include some other methods in general practice and some of the work that has recently appeared.

Water.—In estimating the volatile matter in soaps that are made from the drying or semi-drying oils, there is considerable danger of oxidation when the material is heated in the air-bath at a temperature of 100°. Even with soaps made from other stock, evidences of oxidation are easily detected if the heating is prolonged. Where this trouble is serious, the soap sample, prepared as suggested in Vol. II, should be dried to constant weight in a vacuum oven. R. M. Fitzpatrick[1] estimates the moisture by dissolving the soap in absolute alcohol, filtering, treating the filtrate with anhydrous sodium sulphate, refiltering, then evaporating the alcohol on the water-bath and finally drying in a steam oven. The moisture is calculated from the weight of alcohol insoluble matter and anhydrous soap found.

It is to be remembered that other substances besides water are volatile at a temperature of 100°, including alcohol, naphtha, benzene or other similar compounds that may have been added to the soap. Soap powders containing considerable quantities of sodium hydrogen carbonate, when heated, will suffer considerable loss of carbon dioxide which will have to be estimated and allowed for.

Alcohol, if present, may be estimated by distilling and determining the sp. gr. of the distillate (Vol. II, p. 424). When volatile solvents, immiscible with water, are present in considerable quantity, they may be estimated by distilling a quantity of the soap with steam, until oily drops no longer condense with the water. The distillate is collected in a flask with a narrow graduated neck, into which the oily distillate is finally floated and its volume read off and the weight calculated from the sp. gr. When foaming interferes, the soap may be precipitated by a heavy metal salt before distillation. Moisture may be estimated in such cases by distilling the soap with toluene or other suitable compound until no more water comes over and reading off the volume of water in the distillate.

[1] *Chem. News*, 1911, 104, 247.

Separation of Substances Soluble in Petroleum Ether.—The dried soap may be extracted directly with petroleum ether (Vol. II, p. 424), but since this extraction requires considerable time and care to insure its being complete, it is frequently more satisfactory to extract a solution of the soap (Vol. II, p. 430). However, as soap is very readily hydrolysed in water it is not permissible to use this solvent alone, as considerable quantities of fatty acids would be extracted besides the ether-soluble compounds existing free in the soap. It will be found that if a solvent consisting of about equal parts of water and alcohol be used, the hydrolysis of the soap will be so slight that no serious error will be introduced.[1] The solution obtained should be extracted by shaking out with several portions of petroleum ether. If troublesome emulsions are formed, they can usually be broken by the addition of a little more alcohol. Naturally this extraction gives both the unsaponified and unsaponifiable matter present. These may be separated by making an alcoholic potash or soda saponification of the residue obtained on the evaporation of petroleum ether, then extracting a second time, using the same precautions as above. This will give the unsaponifiable matter only (Vol. II, p. 426).

Estimation of Fatty Acids.—Instead of estimating the fatty acids by the cake method (Vol. II, p. 430) some analysts, after having decomposed the soap, prefer to dissolve the layer of acids in petroleum ether, drawing off the acid water by means of a separating funnel and filtering the ether solution into a tared flask or beaker. If the filter has previously been saturated with the petroleum ether and is kept saturated during the operation, any small quantities of the aqueous solution that may be accidentally transferred to the filter will be held back. After the solution has been extracted with several small portions of the petroleum ether and the funnel and filter carefully washed with the same, the solvent is evaporated and the acids weighed directly. A convenient method of driving off the last traces of petroleum ether is to heat the acids on a steam-bath under a moderate current of air directed into the flask or beaker from a suitable nozzle. It will be found that the acids may be brought to a more nearly constant weight by this method than by heating in an air-bath. The danger of oxidation of acids from the drying oils by this method should be noted and the use of a vacuum oven is recommended in such cases.

Whatever method is used in estimating the fatty acids, the danger of volatilisation must be considered. In fats having a saponification value of about 200, this danger is comparatively slight when reasonable care is used, but with fats containing acids of low molecular weight the loss may be very appreciable. Moreover in soaps that have become rancid with the development of a high free acidity, probably due to the decomposition of fat, volatile acids are present to a very considerable extent. Such a loss, if occurring,

[1] D. Holde, *Zeit. Elektrochem.*, 1910, 16, 436.

may be estimated by taking the fatty acids after weighing, dissolving them in neutral alcohol and titrating, then calculating the combined alkali from this titration. If the results so obtained are less than the combined alkali as estimated by other methods, the indication is that acids have been volatilised. This loss may also be checked by making two extractions, in one of which the acids are weighed and then titrated, whilst in the other the titration is made directly and a correction made in the weight for any loss indicated by a difference of titrations.[1]

It is almost impossible to separate or even extract the fatty acid from the decomposed mass obtained from soaps that contain large quanties of fillers insoluble in water or sodium silicate, which gives a gelatinous precipitate of silicic acid when decomposed with acid. When such difficulties arise, the best procedure is to separate the soap from such material by dissolving in alcohol, filtering and washing the matter insoluble in alcohol carefully so as to insure the removal of all soap. The alcohol is then evaporated and the fatty acids estimated in the purified soap obtained. Several methods have been proposed in which the soap is decomposed and the fatty acids collected in a narrow graduated tube, the volume read off and the weight calculated from the gravity. The method is rapid and with suitable apparatus quite accurate.[2] For a more detailed comparison and discussion of various methods of fatty acid estimation, see G. Fendler and L. Frank.[3]

Examination of Fatty Acids.—In the examination of fatty acids separated from a soap as described in Vol. II, p. 22, with the object of obtaining some idea as to their source, it is to be remembered that in the last few years fats from various sources hardened by hydrogenation have appeared on the market and are more or less extensively used in the manufacture of soap. Since the hardening of various oils converts the oleins and other unsaturated acids or their esters more or less completely into the corresponding saturated compounds, the characteristics of the fat are entirely changed. This is probably most noticed in the reduction in the iodine value and the rise in melting point or titer. Valuable information may, however, be obtained by the examination of the acids, as to the probable action of the soap in use and possibly as to the nature of fats required to make a similar product, but it would be very difficult to form even an approximate idea of the source of fats that have been used if they have been hydrogenated.

Estimation of Free Alkali.—When estimating the free caustic alkali by dissolving the soap in strong alcohol (Vol. II, p. 438), care should be taken to protect the solution from carbon dioxide or other acid fumes, as small quantities of alkali are easily neutralised in this way and so lost. The filter that is to be used should likewise first be neutralised by passing hot alcohol

[1] A. Besson, *Chem. Zeit.*, 1914, **38**, 645 and 686.
[2] O. Schutte, *Seifensieder Ztg.*, 1913, **40**, 551.
[3] *Zeit. angew. Chem.*, 1909, **22**, 252 and 541.

of a slightly alkaline reaction through it until that coming through remains just alkaline.

Another method of estimating free alkali, sometimes used, is to make an aqueous solution of the soap, separate the soap by the addition of salt, filter off and wash the curd with saturated brine. To the filtrate barium chloride is added to precipitate the carbonates. The solution is again filtered and the free alkali hydroxide titrated in the filtrate. The precipitate of barium carbonate on the filter may be dissolved in standard acid, the excess titrated and the free carbonate calculated from the quantity of acid absorbed. If the free alkali hydroxide only is desired, both the soap and the carbonate may be pecipitated directly from the aqueous solution with an excess of barium chloride and the hydroxide titrated in the filtrate.

Both methods may be refined if the most accurate work is desired. For a further comparison see E. Borshard and W. Huggenberg.[1]

Matter Insoluble in Alcohol.—This will, as stated in Vol. II, p. 438, be composed of the carbonate, silicate, borate and like alkaline salts of soda together with fillers insoluble in water. Frequently the only data required will be the weight of the insoluble portion in alcohol together with its total alkalinity. However, if a separate estimation of the various components is desired, it may be made as previously suggested (Vol. II, p. 441).

It is usually preferable to separate the water-soluble alkali from any insoluble filler that may be present and estimate the various alkalies in aliquots of the aqueous extract.

In this estimation, the variable composition of sodium silicate must be taken into consideration. This variation depends upon the composition of the original silicate and the amount of alkali used in the manufacture of the soap, as any excess of free caustic alkali used will tend to be absorbed by the silicate filler, causing the disappearance of free caustic and the formation of a more alkaline silicate. On this account, soaps containing an appreciable quantity of silicate filler usually show little or no free caustic alkalinity. Likewise since silicate of soda can be decomposed by carbon dioxide, soaps, especially in the chip form, that have contained large quantities of silicate of soda may have absorbed sufficient carbon dioxide during long exposure to the air to decompose the silicate more or less completely. In such cases, the quantity of carbonate will be increased and a corresponding quantity of free silicic acid found in the water insoluble portion. Since the combination of soda and silicic acid is so variable, it is preferable to determine the total alkalinity by titration, using methyl-orange and to determine the carbon dioxide, silicic acid and boric anhydride, if present, separately. After combining the proper quantity of soda with the carbon dioxide and boric acid found, the remaining sodium oxide and the silicon dioxide are reported as soda and silica combined as silicate, without attempting to use a definite formula for the silicate.

[1] *Zeit. angew. Chem.*, 1914, 27, 11.

Poetschke[1] has found that sodium borate is not entirely insoluble in alcohol and therefore recommends that the borax be estimated by fusing 10 grm. of the soap with 2 grm. of sodium carbonate and 0.15 grm. of fine silica. After disintegrating the fused product with boiling water, it is acidified with 20 c.c. of 1 : 1 hydrochloric acid, heated nearly to boiling and treated with slight excess of dry calcium carbonate. After boiling for 10 minutes under a reflux apparatus, the liquid is filtered and the filter washed, keeping the volume under 100 c.c. The filtrate is again boiled under the reflux, with the addition of a very small amount of calcium carbonate and cooled under partial vacuum by attaching a suction pump to the top of the reflux. Glycerin is then added to the cooled filtrate and the boric acid titrated. When an end point is obtained, more glycerin is added to see if it is permanent. 1 c.c. of N-solution is equivalent to 0.0505 grm. of anhydrous borax or 0.0955 grm. of borax crystallised with 10 molecules of water.

Fillers Insoluble in Water.—Although the soap may be dissolved directly in water and filtered (Vol. II, p. 429), the filtration of such a solution is usually very difficult and it is usually more convenient to separate the matter insoluble in water and alcohol together, as above, and weigh the portion insoluble in water after making the extract for the estimation of the alkaline carbonates, etc.

Estimation of Special Constituents.—Formaldehyde is present in a number of medicinal soap preparations and may be estimated as follows. The soap is dissolved in 4 or 5 times its weight of water and the soap precipitated either with barium chloride or sulphuric acid, filtered and made up to some definite volume. The formaldehyde is estimated in an aliquot of the filtrate by titrating by the iodometric method (Vol. I, p. 261).[2]

Peroxide soaps or powders are frequently met with in which the peroxygen component may be sodium perborate, percarbonate or the peroxide of some heavy metal, the nature of which will have been determined in the course of analysis. The available oxygen of such a soap may be estimated by dissolving the soap in water and decomposing with acid, care being taken that the solution is kept cool and sufficiently dilute to prevent the peroxide liberated from being decomposed. The acids are filtered off, using kieselguhr, if necessary, to obtain a clear filtrate, washing the residue and making up to volume. To an aliquot of the filtrate, potassium iodide is added and the liberated iodine titrated with standard sodium thiosulphate solution, or if preferred, the peroxide in the filtrate may be determined by acidifying with sulphuric acid and titrating with potassium permanganate,[3] 1 c.c. of an $N/10$ solution in either case being equivalent to 0.0008 grm. oxygen.

Cresols.—For a rapid assay of cresol soap preparations similar to the official disinfecting compounds, M. Seiger[4] proceeds as follows: 20 grm. of

[1] *J. Ind. Eng. Chem.*, 1913, **5**, 645.
[2] O. Alleman, *Zeit. Anal. Chem.*, 1910, **49**, 265; *Seifensieder Ztg.*, 1913, **40**, 49.
[3] F. M. Litterschied and P. B. Guggari, *Chem. Zeit.*, 1910, **37**, 677 and 690.
[4] *Seifensieder Ztg.*, 1911, **38**, 986.

solution with the addition of 500 c.c. of water is twice evaporated to dryness to drive off the cresol. The residue is dissolved in 40 c.c. of water, transferred to a 160 c.c. graduated cylinder and decomposed by adding 5 grm. of sodium chloride and 10 c.c. of strong hydrochloric acid. 20 grm. of petroleum ether are added and the whole is shaken up and allowed to separate. 20 c.c. subtracted from the volume of the ether solution gives the volume of the fatty acid, which multiplied by 0.92 is considered the weight. Another 20 grm. of the original compound are diluted with 20 c.c. of water and treated exactly as above without previously evaporating. In this case, the upper layer consists of the cresols and fatty acid. The factor 1.04 is used to convert the volume of the cresols to the weight.

Soap Powders, Scouring Powders and Scouring Soaps.—The general methods of soap analysis will usually apply to products of the above class, but since the proportions of the various constituents are entirely different, precautions must sometimes be taken. In soap powders the percentage of soap is frequently quite low, the bulk of the powder being composed of sodium carbonate with water of crystallisation. In such cases, estimation of moisture, the weight of the alcohol extract, considered as true soap, and the alkalinity of the alcohol insoluble portion, calculated as sodium carbonate, may give all the data required. A qualitative examination of the matter insoluble in alcohol should be made, as various other alkalies may have been used, including sodium hydrogen carbonate, silicate, aluminate, triphosphate and borate. Potash salts are seldom found except in soft soaps both on account of their greater cost to the manufacturer and their hygroscopic nature.

Scouring powders usually consist of a large percentage of abrasive material with a comparatively small quantity of soap, moisture and alkali. Frequently a short method of assay, similar to that suggested above but including an estimation of water insoluble matter, would answer all requirements. A microscopic and more or less practical examination of the abrasive material might give valuable information. The abrasive material should be sharp and have decided mechanical cleansing value, but should not be too hard or it may be destructive to the surfaces on which it is used. Likewise, it should be practically free from extremely fine or clay-like impurities.

Scouring soaps are similar in their general composition to the powders described above, except they are in bar form. The quantity of abrasive material in such soap varies from an ordinary household bar soap containing a few per cent., to a scouring brick that contains only enough soap or other agent to act as a binding material.

The following table gives a list of typical analyses of various classes of soaps commonly found on the American market.

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Kind of soap	Fatty anhydride	Rosin acids	Combined alkali	Free caustic	Free carbonate	Silicate[1] of soda	Salt	Water insoluble	Glycerin and undetermined	Volatile matter
Milled toilet...........	79.39	......	9.24	0.08	0.37	......	0.12	......	0.38	10.42
Milled toilet...........	68.51	......	7.98	0.12	0.55	......	0.18	11.21[4]	0.43	11.02
Floating..............	63.24	......	8.23	0.02	0.33	......	0.35	......	0.52	27.31
Cocoa.................	57.95	......	8.58	0.01	0.17	......	0.21	......	6.12	26.96
Transparent[2].........	43.33	......	5.50	0.12	0.73	......	0.60	......	10.51	24.58
Household bar ,yellow..	41.56	18.83	6.30	0.04	1.87	2.38	0.28	trace	0.45	28.29
Household bar, yellow..	28.28	20.45	5.10	0.02	2.34	3.18	0.40	11.28[5]	0.38	28.57
Household bar, white...	43.48	......	5.64	0.02	2.05	11.11	0.23	......	0.98	36.49
Laundry chip..........	78.99	......	9.20	0.22	0.64	......	0.10	......	0.44	10.41
Laundry chip..........	57.54	......	6.82	0.03	3.95	7.91	0.18	......	0.70	22.87
Soft potash[5]...........	40.25	......	6.92	0.06	1.19	......	0.45	......	5.92	45.21

ERRATA IN VOL. II.

Page 373, in heading of 6th column of table B, "Insomeric" should read "Isomeric.'
Page 381, line 12, for "212" read "213." Line 14, for "deducted" read "deduced."
Page 401, line 14 for "testing" read "heating."

[1] Silicate of soda calculated on the basis of 1 part of Na_2O combining with 3.14 parts of SiO_2.
[2] Contained 14.63 % sugar.
[3] All alkalies calculated as potassium compounds.
[4] Water insoluble material was starch.
[5] Water insoluble material was silica.

GLYCEROL.

By WILLIAM A. DAVIS.

Specific Gravity.—Redeterminations have been made by Kailan[1] of the sp. gr. of anhydrous glycerol at temperatures between 14 and 20°; $d_4^{14°}$ = 1.26414 and $d_4^{20°}$ = 1.26082. The following equation gives the relation existing between sp. gr. and temperature:

$$d_4^{t°} = 1.26413 + (15 + t)0.000632$$

A series of determinations of the boiling point of anhydrous glycerol under diminished pressure (32 to 9 mm.) was carried out, and values corroborating the vapour tensions given by Richardson[2] obtained. Experiments on the hygroscopicity of glycerin showed that in an atmosphere of average moisture content equilibrium is reached with a mixture containing about 80% of glycerol. Concentrated alcohol absorbs water about 4 times as rapidly as concentrated glycerol.

Analysis of Crude Glycerol.[3]—(International Standard Methods, 1911.)— The valuation of crude glycerol has in recent years assumed greater commercial importance owing to the increased value of the commodity. The want of uniformity in the methods and processes of analysis, together with the irregularity of the results obtained, emphasised the desirability for the standardisation of crude glycerol analysis; with this object in view, committees were formed in America, France, Germany and Great Britain. The methods detailed in this report have the unanimous support of each of the above committees, and are strongly recommended by them as International Standards.

Sampling.—The most satisfactory method available for sampling crude glycerol liable to contain suspended matter, or which is liable to deposit salt on settling, is to have the glycerol sampled by a mutually approved sampler as soon as possible after it is filled into drums, but in any case before any separation of salts has taken place. In such cases he shall sample with a sectional sampler (a suitable sampling apparatus is described in an appendix to the report), then seal the drums, brand them with a number for identification and keep a record of the brand number. The presence of any visible salt or other suspended matter is to be noted by the sampler and a report

[1] *Zeit. anal. Chem.*, 1912, **51**, 81.
[2] *Trans.*, **49**, 764.
[3] *Analyst*, 1911, **36**, 314.

of same made in his certificate, together with the temperature of the glycerol. Each drum must be sampled. Glycerol which has deposited salt or other matters cannot be accurately sampled from the drums, but an approximate sample can be obtained by means of the sectional sampler, which will allow a complete vertical section of the glycerol to be taken, including any deposit

Analysis.—(1) *Estimation of Free Alkali Hydroxide.*—Weigh 20 grm. of the sample into a 100 c.c. flask, dilute with approximately 50 c.c. of freshly boiled distilled water, add an excess of neutral barium chloride solution, 1 c.c. of phenolphthaleïn solution, make up to the mark and mix. Allow the precipitate to settle, draw off 50 c.c. of the clear liquid, and titrate with normal acid ($N/1$). Calculate to percentage of Na_2O existing as alkali hydroxide.

(2) *Estimation of Ash and Total Alkalinity.*—Weigh 2 to 5 grm. of the sample in a platinum dish, burn off the glycerol over a luminous Argand burner or other source of heat giving a low flame temperature, the temperature being kept low to avoid volatilisation and the formation of sulphides. When the mass is charred to the point that water will not become coloured by soluble organic matter, lixiviate with hot distilled water, filter, wash and ignite the residue in the platinum dish. Return the filtrate and washings to the dish, evaporate, and carefully ignite without fusion. Weigh the ash.

Dissolve the ash in distilled water and titrate total alkalinity, using as indicator, methyl-orange cold or litmus boiling.

(3) *Estimation of Alkali present as Carbonate.*—Take 10 grm. of the sample, dilute with 50 c.c. of distilled water, add sufficient $N/1$ acid to neutralise the total alkali found at (2), boil under a reflux condenser for 15 to 20 minutes, wash down the condenser tube with distilled water free from carbon dioxide, and titrate back with $N/1$ sodium hydroxide using phenolphthaleïn as indicator. Calculate the percentage of Na_2O. Deduct the Na_2O found in (1). The difference is the percentage of Na_2O existing as carbonate.

(4) *Alkali combined with Organic Acids.*—The sum of the percentages of Na_2O found at (1) and (3) deducted from the percentage found at (2) is a measure of the Na_2O or other alkali combined with organic acids.

(5) *Determination of Acidity.*—Take 10 grm. of the sample, dilute with 50 c.c. of distilled water free from carbon dioxide, and titrate with $N/1$ sodium hydroxide and phenolphthaleïn. Express in terms of Na_2O required to neutralise 100 grm.

(6) *Estimation of Total Residue at* 160° *C.*—For this estimation the crude glycerol should be slightly alkaline with sodium carbonate, not exceeding the equivalent of 0.2 % Na_2O, in order to prevent loss of organic acids. To avoid formation of polyglycerols, this alkalinity must not be exceeded.

Preparation of Glycerol.—10 grm. of the sample are weighed into a 100 c.c. flask diluted with water and the calculated quantity of $N/1$ hydrochloric acid

or sodium carbonate added to give the required degree of alkalinity. The flask is filled to 100 c.c., the contents mixed and 10 c.c. measured into a weighed Petri or similar dish 2.5 in. diameter and 0.5 in. deep, which should have a flat bottom. In the case of crude glycerols abnormally high in organic residue, a less quantity is to be evaporated, so that the weight of organic residue does not materially exceed 30 to 40 mg.

Evaporation of the Glycerol.—The dish is placed on a water-bath (the top of the 160° C. oven acts equally well) until most of the water has evaporated. From this point the evaporation is effected in the oven. Satisfactory results are obtained in an oven[1] measuring 12 in. cube, having an iron plate $\frac{3}{4}$ in. thick lying on the bottom to distribute the heat. Strips of asbestos millboard are placed on a shelf halfway up the oven. On these strips the dish containing the glycerol is placed.

If the temperature of the oven has been adjusted to 160° C. with the door closed, a temperature of 130° to 140° C. can be readily maintained with the door partially open, and the glycerol, or most of it, should be evaporated off at this temperature. When only a slight vapour is seen to come off, the dish is removed and allowed to cool.

0.5 to 1 c.c. of water is added and by a rotary motion the residue brought wholly or nearly into solution. The dish is then allowed to remain on a water-bath or top of the oven until the excess of water has evaporated and the residue is in such a condition that on returning to the oven at 160° C. it will not spit. The time taken up to this point cannot be given definitely, nor is it important. Usually 2 to 3 hours are required. From this point, however, the schedule of time must be strictly adhered to. The dish is allowed to remain in the oven, the temperature of which is carefully maintained at 160° C. for 1 hour, when it is removed, cooled, the residue treated with water and the water evaporated as before. The residue is then subjected to a second baking of 1 hour, after which the dish is allowed to cool in a desiccator over sulphuric acid and weighed. The treatment with water, etc., is repeated until a constant loss of 1 to 1.5 mg. per hour is obtained.

Corrections to be Applied to the Weight of the Total Residue.—In the case of acid glycerol, a correction must be made for the alkali added. 1 c.c. $N/1$ alkali represents an addition of 0.022 grm. In the case of alkaline crudes a correction should be made for the acid added. Deduct the increase in weight due to the conversion of the sodium hydroxide and carbonate to NaCl. The corrected weight, multiplied by 100, gives the percentage of *total residue at* 160° C.

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[1] Grimwood (*J. Soc. Chem. Ind.*, 1913, 32, 1040) states that the type of oven specified here has proved quite unsatisfactory; a variation of 16° in the temperature was found on one shelf and between two shelves a maximum variation of 45° was experienced. Grimwood has described an electrically heated oven for the purpose of these estimations, which was designed so as to ensure uniformity of temperature throughout. An arrangement is provided to eliminate the glycerol vapour rapidly from the oven by means of an air blast, so as to facilitate the evaporation.

When a limited number of analyses have to be made it is probably best to use a Meyer vapour bath or an Abati oven (Vol. I, p. 69) heated by the vapour of a suitably chosen fraction of high boiling petroleum or turpentine; in this way the proper temperature can easily be ensured.

Preserve the total residue for the estimation of the non-volatile ace-tylisable impurities.

(7) *Organic Residue.*—Subtract the ash from the total residue at 160° C. Report as organic residue at 160° C. (*Note.*—It should be noted that alka-line salts of organic acids are converted to carbonates on ignition, and that the CO_2 radicle thus derived is not included in the organic residue.)

(8) *Moisture.*—This test is based on the fact that glycerol can be com-pletely freed from water by allowing it to stand *in vacuo* over sulphuric acid or phosphoric anhydride.

2 to 3 grm. of very pure bulky asbestos, freed from acid soluble material, which has been previously dried in a water oven are placed in a small stoppered weighing bottle of about 15 c.c. capacity. The weighing bottle is kept in a vacuum desiccator furnished with a supply of concentrated sulphuric acid, under a pressure equivalent to 1 to 2 mm. of mercury, until constant in weight. From 1 to 1.5 grm. of the sample is then carefully dropped on the asbestos in such a way that it will be all absorbed. The weight is again taken, and the bottle replaced in the desiccator under 1 to 2 mm. pressure until constant in weight. At 15° C. the weight is constant in about 48 hours. At lower temperatures the test is prolonged.

The sulphuric acid in the desiccator must be frequently renewed.

Acetin Process for Glycerol Estimation.—This process is the one agreed upon at a Conference of Delegates from the American, British, French and German Committees, and has been confirmed by each of the above Committees as giving results nearer to the truth on crudes in general, and is the process to be used (if applicable) whenever only one method is em-ployed. On pure glycerols the results are identical with those of the bichro-mate process. For the application of this process the crude glycerol should not contain over 50% of water.

The following reagents are required:

(1) *Best Acetic Anhydride.*—This should be carefully selected. A good sample must not require more than 0.1 c.c. normal sodium hydroxide for saponfication of the impurities when a blank is run on 7.5 c.c. Only a slight colour should develop during digestion of the blank.

(2) *Pure Fused Sodium Acetate.*—The purchased salt is again completely fused in a platinum, silica, or nickel dish, avoiding charring, powdered quickly, and kept in a stoppered bottle or in a desiccator. It is most important that the sodium acetate be anhydrous.

(3) *A Solution of Sodium Hydroxide for Neutralising, of about N/1 Strength, Free from Carbonate.*—This can be readily made by dissolving pure sodium hydroxide in its own weight of water (preferably water free from carbon di-oxide), and allowing to settle until clear, or filtering through an asbestos or paper filter. The clear solution is diluted with water free from carbon diox-ide to the strength required.

(4) *N/1 Sodium Hydroxide, Free from Carbonate.*—Prepared as above, and carefully standardised.

Some sodium hydroxide solutions show a marked diminution in strength after being boiled; such solutions should be rejected.

(5) *N/1 Acid.*—Carefully standardised.

(6) *Phenolphthalein Solution.*—0.5% phenolphthalein in alcohol and neutralised.

The Method.—Into a narrow-mouthed flask (preferably round-bottomed) of capacity about 120 c.c., which has been thoroughly cleaned and dried, weigh accurately and as rapidly as possible 1.25 to 1.5 grm. of the glycerol. Add first about 3 grm. of the anhydrous sodium acetate, then 7.5 c.c. of the acetic anhydride and connect the flask with an upright Liebig condenser. For convenience the inner tube of this condenser should not be over 50 cm. long and 9 to 10 mm. inside.

The flask is connected to the condenser by either a ground glass joint (preferably) or a rubber stopper. If a rubber stopper is used, it should have had a preliminary treatment with hot acetic anhydride vapour.

Heat the contents and keep just boiling for 1 hour, taking precautions to prevent the salts drying on the sides of the flask.

Allow the flask to cool somewhat, and through the condenser tube add 50 c.c. of the carbon-dioxide-free distilled water, heated to about 80° C., taking care that the flask is not loosened from the condenser. The object of cooling is to avoid any sudden rush of vapours from the flask on adding the water and to avoid breaking the flask. Time is saved by adding the water before the contents of the flask solidify, but the contents may be allowed to solidify and the test proceeded with the next day without detriment. The contents of the flask may be warmed to, but must not exceed, 80° C. until solution is complete except a few dark flocks representing organic impurities in the crude. By giving the flask a rotatory motion, solution is more quickly effected. Cool the flask and contents without loosening from condenser. When quite cold wash down the inside of the condenser tube, detach the flask, wash the stopper or ground glass connection into the flask, and filter contents of flask through an acid-washed filter into a Jena glass flask of about 1 litre capacity. Wash thoroughly with cold distilled water free from carbon dioxide. Add 2 c.c. of phenolphthalein solution (6), then run in sodium hydroxide solution (3) or (4) until a faint pinkish-yellow colour appears throughout the solution. This neutralisation must be done most carefully. The alkali should be run down the sides of the flask, the contents of which are kept rapidly swirling with occasional agitation or change of motion until the solution is nearly neutralised, as indicated by the slower disappearance of the colour developed locally by the alkali running into the mixture. When this point is reached the sides of the flask are washed down with carbon-

dioxide-free water and the alkali subsequently added drop by drop, mixing after each drop, until the desired tint is obtained.

Now run in from a burette 50 c.c. or a calculated excess of $N/1$ sodium hydroxide (4), and note carefully the exact amount. Boil gently for 15 minutes, the flask being fitted with a glass tube acting as a partial condenser; cool as quickly as possible, and titrate excess of sodium hydroxide with $N/1$ acid (5) until the pinkish-yellow or chosen end-point colour just remains. A further addition of the indicator at this point will cause a return of the pinkish colour; this must be neglected, and the first end point taken.

From the $N/1$ sodium hydroxide consumed calculate the precentage of glycerol after making the correction for the blank test described below:

1 c.c. of $N/1$ sodium hydroxide = 0.03069 grm. of glycerol

The coefficient of expansion for normal solutions is approximaely 0.00033 per c.c. for each degree C. A correction should be made on this account if necessary.

Blank Test.—As the acetic anhydride and sodium acetate may contain impurities which affect the result, it is necessary to make a blank test, using the same quantities of acetic anhydride and sodium acetate as in the analysis. After neutralising the acetic acid, it is not necessary to add more than 5 c.c. of the $N/1$ alkali (4), as that represents the excess of alkali usually left after saponification of the triacetin in the glycerol determination.

Estimation of the Glycerol Value of the Acetylisable Impurities.—The total residue at 160° C. is dissolved in 1 or 2 c.c. of water, washed into a clean acetylating flask, 120 c.c. capacity, and the water evaporated. Now add anhydrous sodium acetate and proceed as in the glycerol determination before described. Calculate the result to glycerol.

Analysis of Acetic Anhydride.—Into a weighed stoppered vessel, containing 10 to 20 c.c. of water, run about 2 c.c. of the anhydride, replace stopper and weigh; allow to stand, with occasional shaking, for several hours, till all anhydride is hydrolysed; then dilute to about 200 c.c., add phenolphthaleïn, and titrate with $N/1$ sodium hydroxide. This gives the total acidity due to free acetic acid and acid formed from anhydride.

Into a stoppered weighing-bottle containing a known weight of recently distilled aniline (from 10 to 20 c.c.) measure about 2 c.c. of the sample, stopper, mix, allow to cool and weigh. Wash contents into about 200 c.c. of cold water and titrate acidity as before. This yields the acidity due to the original, preformed, acetic acid plus one-half the acid due to anhydride (the other half having formed acetanilide); subtract the second result from the first (both calculated for 100 grm.) and double result, obtaining c.c. of $N/1$ sodium hydroxide per 100 grm. sample. 1 c.c. of sodium hydroxide equals 0.0510 grm. of acetic anhydride.

Dichromate Process for Glycerol Estimation.—MATERIALS RE-
QUIRED.—(a) *Pure potassium dichromate* powdered and dried in air free from
dust or organic vapours, at 110° to 120° C. This is taken as the standard.

(b) *Dilute Dichromate Solution.*—7.4564 grm. of the above dichromate
(a) are dissolved in distilled water and the solution made up to 1 litre at
15.5°.

(c) *Ferrous Ammonium Sulphate.*—Dissolve 3.7282 grm. of potassium
dichromate (a) in 50 c.c. of water. Add 50 c.c. of 50% (by volume) sul-
phuric acid and to the cold undiluted solution add from a weighing bottle a
moderate excess of the ferrous ammonium sulphate, and titrate back with the
dilute dichromate (b). Calculate the value of the ferrous salt in terms of
dichromate.

(d) *Silver Carbonate.*—This is prepared as required for each test from
140 c.c. of 0.5% silver sulphate solution by precipitation with about
4.9 c.c. $N/1$ sodium carbonate solution (a little less than the calculated quan-
tity of $N/1$ sodium carbonate should be used; any excess of alkali carbonate
prevents rapid settling). Settle, decant and wash once by decantation.

(e) *Subacetate of Lead.*—Boil a pure 10% lead acetate solution with
an excess of litharge for 1 hour, keeping the volume constant and filter
while hot. Disregard any precipitate which subsequently forms. Preserve
out of contact with carbon dioxide.

(f) *Potassium Ferricyanide.*—A very dilute solution containing about
0.1%.

The Method.—Weigh 20 grm. of the glycerol, dilute to 250 c.c. and take
25 c.c. Add the silver carbonate, allow to stand, with occasional agitation,
for about 10 minutes and add a slight excess (about 5 c.c. in most cases) of
the basic lead acetate (e), allow to stand a few minutes, dilute with distilled
water to 100 c.c. and then add 0.15 c.c. to compensate for the volume of the
precipitate, mix thoroughly, filter through an air-dry filter into a suitable
narrow-mouthed vessel, rejecting the first 10 c.c., and return filtrate if not
clear and bright. Test a portion of the filtrate with a little basic lead acetate,
which should produce no further precipitate. (In the great majority of cases
5 c.c. is ample.) Occasionally a crude glycerol will be found requiring more
and in this case another aliquot of 25 c.c. of the dilute glycerol should be
taken and purified with 6 c.c. of the basic lead acetate. Care must be
taken to avoid a marked excess of basic acetate.

Measure off 25 c.c. of the clear filtrate into a glass flask or beaker (pre-
viously cleaned with potassium dichromate and sulphuric acid). Add 12
drops of sulphuric acid (1 : 4) to precipitate the small excess of lead as sul-
phate. Add 3.7282 grm. of the powdered potassium dichromate (a).
Rinse down the dichromate with 25 c.c. of water and leave with occasional
shaking until all the dichromate is dissolved (no reduction will take place).

Now add 50 c.c. of 50% sulphuric acid (by volume) and immerse the

vessel in boiling water for 2 hours and keep protected from dust and organic vapours, such as alcohol, until the titration is completed. Add from a weighing bottle a slight excess of the ferrous ammonium sulphate (c), making spot tests on a porcelain plate with the potassium ferricyanide (f). Titrate back with the dilute dichromate. From the amount of dichromate reduced calculate the percentage of glycerol.

1 grm. glycerol equals 7.4564 grm. dichromate.
1 grm. dichromate equals 0.13411 grm. glycerol.

Notes.—(1) It is important that the concentration of acid in the oxidation mixture and the time of oxidation should be strictly adhered to.

(2) Before the dichromate is added to the glycerol solution it is essential that the slight excess of lead be precipitated with sulphuric acid as stipulated in the process.

(3) For "crudes" practically free from chlorides the quantity of silver carbonate may be reduced to one-fifth and the basic lead acetate to 0.5 c.c.

(4) It is sometimes advisable to add a little potassium sulphate to insure a clear filtrate.

Instructions for Calculating Actual Glycerol Content.—(1) Determine the apparent percentage of glycerol in the sample by the acetin process as described. The result will include acetylisable impurities, if any be present.

(2) Determine the total residue at 160° C.

(3) Determine the acetin value of the residue at (2) in terms of glycerol.

(4) Deduct the result found at (3) from the percentage obtained at (1) and report this corrected figure as glycerol. If volatile acetylisable impurities are present, these are included in this figure.

Notes and Recommendations.—Experience has shown that in crude glycerol of good commercial quality the sum of water, total residue at 160° C. and corrected acetin results comes to within 0.5 of 100. Further in such "crudes" the dichromate result agrees with the uncorrected acetin result to within 1%.

In the event of greater differences being found, impurities, such as polyglycerols or trimethyleneglycol, are present. Trimethyleneglycol is more volatile than glycerol; it can therefore be concentrated by fractional distillation. An approximation to the quantity can be obtained from the divergence between the acetin and dichromate results of such distillates, trimethylene-glycol showing by the former method 80.69%, and by the latter 138.3%, expressed as glycerol.

In valuing crude glycerol for certain purposes it is necessary to ascertain the approximate proportion of arsenic, sulphides, sulphites and thiosulphates. The methods for detecting and determining these impurities have not formed the subject of this investigation.

Recommendations by Executive Committee.—If the non-volatile organic residue at 160° C. in the case of a soap lye "crude" be over 2.5%—*i.e.*, when

not corrected for carbon dioxide in the ash—then the residue shall be examined by the acetin method, and any excess of glycerol found over 0.5% shall be deducted from the acetin figure.

In the case of saponification, distillation and similar glycerol, the limit of organic residue which should be passed without further examination shall be fixed at 1%. In the event of the sample containing more than 1%, the organic residue must be acetylated and any glycerol found (after making the deduction of 0.5%) shall be deducted from the percentage of glycerol found by the acetin test.

British Standard Specifications for Crude Glycerins.—The following standard specifications were drawn up by the British Executive Committee on crude glycerin analysis and approved at a general meeting of crude glycerin makers, buyers and brokers held in London, on Oct. 3, 1912.

Soap Lyes Crude Glycerin.—Analysis to be made in accordance with the International Standard Methods (given above):

Glycerol.—The standard shall be 80% of glycerol. Any crude glycerin tendered which tests 81% of glycerol or over shall be paid for at a *pro rata* increase, calculated as from the standard of 80%. Any crude glycerin which tests under 80% of glycerol, but is 78% or over shall be subject to a reduction of 1½ times the shortage, calculated at a *pro rata* price as from 80%. If the test falls below 78% the buyer shall have the right of rejection.

Ash.—The standard shall be 10%. In the event of the percentage of ash exceeding 10%, but not exceeding 10.5% a percentage deduction shall be made for the excess calculated as from 10% at *pro rata* price and if the percentage of ash exceeds 10.5% but does not exceed 11% an additional percentage deduction shall be made equal to double the amount in excess of 10.5%. If the amount of ash exceeds 11% the buyer shall have the right of rejection.

Organic Residue.—The standard shall be 3%. A percentage deduction shall be made of 3 times the amount in excess of the standard of 3% calculated at *pro rata* price. The buyer shall have the right to reject any parcel which tests over 3.75%.

Saponification Crude Glycerin.—Analysis to be made in accordance with the International Standard Methods, 1911.

Glycerol.—The standard shall be 88%. Any crude glycerin tendered which tests 89% or over shall be paid for at a *pro rata* increase calculated as from the standard of 88%. Any crude glycerin which tests under 88%, but is 86% or over shall be subject to a reduction of 1½ times the shortage calculated at *pro rata* price as from 88%. If the test falls below 86% the buyer shall have the right of rejection.

Ash.—The standard shall be 0.5%. In the event of the ash exceeding 0.5%, but not exceeding 2.0%, a percentage reduction shall be made equal to double the amount in excess of 0.5%. If the amount of ash exceeds 2.0% the buyer shall have the right to reject the parcel.

Organic Residue.—The standard shall be 1%. A percentage deduction shall be made of twice the amount in excess of the standard of 1%, calculated at *pro rata* price. The buyer shall have the right to reject any parcel which tests over 2%.

Pure Glycerin.—L. F. Kebler and H. C. Fuller[1] have examined a number of samples of commercially pure glycerin in order to ascertain to what extent commercial glycerin complies with the requirements of the U. S. Pharmaco-pœia. Eleven samples were obtained from American manufacturers, whilst 2 were of foreign origin, purchased in the open market. The sp. gr. at 25° ranged from 1.248 to 1.258, all exceeding the Pharmacopœia standard of 1.246. In every case reduction occurred when the samples were submitted to the pharmacopœal test with ammoniacal silver nitrate. This test is therefore regarded as inconclusive and Hager's test[2] is preferred; according to this, 5 c.c. of the glycerin are mixed with 5 c.c. of 26% ammonia and 5 drops of silver nitrate solution and the mixture left in the dark for 15 minutes at the ordinary temperature.

Two of the samples gave a pronounced reduction, 5 a slight colouration, whilst with 6 there was no reduction. The quantity of arsenic ranged from *nil* to 0.75 part per million with the exception of a foreign sample which contained 3.75 parts. On the basis of this examination the authors conclude that glycerin of the best quality should answer to the following requirements: It should be neutral to litmus, leave no ash on ignition and have a sp. gr. of about 1.25 at 25°. It should emit only a slight odour when heated on the water-bath and not give off an unpleasant ethereal or a fruity odour when warmed with alcohol and sulphuric acid. When mixed with an equal volume of sulphuric acid there should be no disagreeable odour nor any colouration deeper than yellow. In Hager's test no colouration, or at most a yellow colouration, should be developed. It should not contain sulphates, chlorides, oxalates, metals or sugars and when mixed with an equal volume of water should not reduce Fehling's solution Arsenic in excess of the limit fixed by the U. S. Pharmocopœia should not be present.

British Pharmacopœia, 1914.—The following are the requirements:

Glycerinum.—Sp. gr. 1.260. Neutral to litmus. An aqueous solution (1 in 10) yields no characteristic reaction for ammonium, chlorides, or sulphates. Assumes when heated not more than a faint yellow but no pink colouration and yields not more than a very slight charred residue and no odour of burnt sugar (absence of sugar), undergoes no darkening in colour when mixed into an equal volume of solution of ammonia and a few drops of solution of silver nitrate, the mixture being kept protected from light and the observations made after the lapse of 5 minutes (absence of formic acid and acrolein). Gently warmed with an equal volume of diluted sulphuric acid, the mixture being vigorously shaken, not more than a faint odour is notice-

[1] *U. S. Dept. Agric., Bureau of Chemistry, Bull.* 150, 1912, pp. 25-35.
[2] *Handbuch Pharm. Praxis.*, 1905, 1, 1221.

able (absence of fatty acids). Shaken with an equal volume of sulphuric acid the mixture being kept cool, not more than a very slight straw colouration is produced (absence of extraneous organic matter). A mixture of 10 c.c. of glycerin with 40 c.c. of water, 1 drop of solution of ammonia and 1 drop of solution of tannic acid, assumes not more than a faint and transient pink or purple colouration (limit of iron). When tested for lead according to the quantitative test described in the British Pharmacopœia, Appendix V, but using 10 grm. in each Nessler glass, no difference is observed upon the addition of the solution of sodium sulphide to one of the solutions (absence of lead); when the foregoing test is repeated, but omitting the addition of solution of ammonia and of solution of potassium cyanide and adding to each solution 1 c.c. of diluted hydrochloric acid, no difference in colour is observed upon the addition of solution of hydrogen sulphide to one of the solutions (absence of copper). Arsenic limit 2 parts per million. No appreciable ash.

Aldehydic Impurities in Glycerol.—The impurities to which the reducing properties of the majority of the better qualities of glycerol are due are derived from acroleïn. According to Bergh[1] glycerol and acroleïn combine in equimolecular proportions forming *glycerol-acrylol*, which possesses the properties of an acetal. It does not reduce Fehling's solution and only slightly reduces ammoniacal silver nitrate. It is slowly dissociated by water, more rapidly on heating and is decomposed by dilute acids. Its presence may be detected by means of *fuchsine* sulphite solution or by its reducing action on Fehling's solution, obtained after liberating the acroleïn by carefully warming with dilute sulphuric acid.

Estimation of Glycerol in Wines.—Beys[2] states that discordant results obtained in estimating glycerol in wine by the usual methods are caused mainly by variations in the quantity of barium hydroxide used to render the sugar insoluble. In a later paper[3] the following process is recommended: The volume of wine taken for analysis is 100 c.c. in the case of dry wines, 50 c.c. in the case of sweet wines and 25 c.c. if the sp. gr. exceed 5° Bé. The wine is neutralised with barium hydroxide and evaporated to a syrup in a platinum dish at a temperature not exceeding 70°. Some sand is added and the mixture extracted with acetone, such a quantity of the latter being first used that the strength of the acetone is not reduced below 95% by admixture with the syrupy residue; after this extraction, which is carried out at a temperature below 56°, the liquid is cooled and filtered and the residue extracted with successive quantities of 40–50 c.c. of acetone until at least 200 c.c. of the filtrate are obtained. Two aliquot portions of the filtrate are evaporated (without boiling). In one of the residues the invert sugar is estimated by Fehling's solution; the other is dissolved in 5 times its weight of water and a quantity of powdered barium hydroxide added as follows:

[1] *Apoth. Zeit.*, 1908, 23, 689.
[2] *Compt. rend.*, 1910, 151, 80.
[3] *Bull. Soc. Chem.*, 1912, 11, 618.

(1) If the weight of the sugar is less than 0.05 grm., a few milligrams of barium hydroxide in excess of the sugar are added.

(2) If the weight of the sugar is between 0.05 and 0.3 grm., an equal weight of barium hydroxide is used.

(3) If the sugar is between 0.3 and 0.5 grm., $\frac{2}{5}$ of its weight of barium hydroxide is added.

The mixture is frequently shaken and after about 30 minutes some sand is added and the glycerol extracted by heating, first with 40 c.c., then 2 or 3 times with 25 c.c. of acetone. The solution after filtration is evaporated at a temperature below 56°, the residue being dried at 60 to 65° and weighed. The glycerin so obtained contains about 5 mg. of impurities which about compensates for loss in evaporation, etc.

Estimation of Glycerol in Fats.—Willstätter and Madinaveitia,[1] state that the drawbacks (due to imcomplete hydrolysis) of the method of Zeisel and Fanto described in Vol. III, p. 461, when applied to fats, are obviated by using hydriodic acid of sp. gr. 1.8, with small quantities of the fat (0.15 to 0.35 grm.). About 0.2 grm. of the glyceride is treated with 10 c.c. of the hydriodic acid (sp. gr. 1.8) in Zeisel and Fanto's apparatus, the mixture being heated at 110–115° until the action starts; the temperature is then kept constant for 20–40 minutes, until the silver solution in the absorption flask becomes clear again, after which the heating is continued for 1 hour at 130–140°. The glycerol is then calculated from the amount of isopropyl iodide as in the original method. Results are given by the authors which show that when the method is carried out in the manner described, it possesses a very considerable degree of accuracy.

ERRATA IN VOL. II.

Page 461, line 11 from bottom, for "AgNo₃" read AgNO₃.
Page 475, line 13 from bottom, after "Mayrhofer," insert "see page 434."
Page 475, line 4 from bottom, for "Wainright" read "Wainwright."

[1] *Ber.*, 1912, 45, 2825.

CHOLESTEROL.

By J. ADDYMAN GARDNER, M. A., F. I. C.

ESTIMATION OF CHOLESTEROL IN ANIMAL TISSUES.

Various methods have been proposed in recent years for the accurate estimation of cholesterol and its esters in tissue extracts, but whatever the method adopted for this purpose, the accuracy of the estimation in the tissue itself must depend ultimately on the thoroughness with which the fats and lipoid substances are extracted from the tissue.

Pflüger[1] long ago showed that it was impossible to extract the whole of the fat from a dried tissue by simply extracting with ether in a Soxhlet apparatus and Dormeyer[2] proposed to get over the difficulty by digesting with pepsin and hydrochloric acid prior to the extraction with ether.

Kumagawa and Suto[3] and Schimidzu[4] find that the most certain method of extracting the whole of the fatty acids and unsaponifiable matter contained in a tissue is to destroy the tissue completely by heating with an alkali. Grigaut[5] carries this out in the following manner: 20 c.c. of blood serum are mixed with 20 c.c. of a solution of sodium hydroxide, containing 400 grm. NaOH per litre, and heated in an autoclave at 110° for 1 hour. In the case of solid tissues 5 to 10 grm. of the fresh tissue are heated in a similar manner with 40 c.c. of the soda solution diluted to half the strength.

The disadvantage of this method is that the cholesterol esters are saponified and therefore cannot be estimated. Kumagawa and Suto have, however, shown that the whole of the fats, etc., can be extracted from a tissue by boiling with absolute alcohol and they describe in their paper a convenient apparatus for the purpose.

For some years the writer has adopted the following method and obtained on the whole satisfactory results, so far as cholesterol is concerned. The *fresh* tissue is minced and ground to a fine pulp with fine sand. It is then mixed with 3 or 4 times its weight of plaster of Paris and allowed to set. The dry mass is then ground up again and extracted with ether in a Soxhlet for 2 or 3 weeks. On the whole it is better, particularly when time is a consideration, to extract first with hot alcohol for 1 hour, and then with ether for a day or so, especially in the case of serum, for in this substance the cholesterol appears to be more firmly held; and the writer now uses this method. In the case of liver, which contains enzymes which hydrolyse cholesterol esters, the

[1] *Archiv f. d. ges. Physiol.*, 1892, 51, 277.
[2] *Arch. f. d. ges. Physiol.*, 61, 341 and 65, 90.
[3] *Biochem. Zeitschr.*, 1904, 4, 186.
[4] *Biochem. Zeitschr.*, 1910, 28, 237–273.
[5] *Le cycle de la Cholestérinémie*, Paris, 1913.

tissue should be extracted as soon as possible after removal from the body, or else treated in such a way as to destroy the enzyme.

Estimation of Cholesterol and Cholesterol Esters in an Extract.

Method of Windaus.—Windaus in 1909[1] discovered that cholesterol, but not esters of cholesterol, readily combines quantitatively with digitonin to form a highly insoluble digitonin-cholesteride, according to the following equation:

$$C_{55}H_{94}O_{28} + C_{27}H_{46}O = C_{82}H_{140}O_{29}$$

Digitonin-cholesteride is insoluble in water, acetone, ether, petroleum ether, ethyl acetate and benzene. 100 c.c. of ethyl alcohol (95%) dissolve at 18° only 0.014 grm., and at 78° C. about 0.16 grm.; 100 c.c. of 50% alcohol at a boiling temperature dissolve 0.03 grm. It is readily soluble in pyridine and slightly in methyl alcohol.

Digitonin-cholesteride readily dissociates on heating in the vapour of boiling xylene. The compound is placed in a paper thimble and suspended in a flask containing boiling xylene. After heating for 15 hours the dissociation is usually complete. The cholesterol dissolves in the xylene and the insoluble digitonin remains in the thimble and can be used again. The cholesterol is readily recovered by distilling off the xylene in steam.

For the quantitative estimation Windaus adopts the following plan:[2]

The ethereal or other extract of a tissue is evaporated and the residue taken up in 30 times its volume of hot 95% alcohol. This solution is treated with a 1% solution of digitonin in hot 90% alcohol so long as a precipitate is produced, care being taken to leave the digitonin in slight excess. After several hours the precipitate is filtered off on a Gooch crucible, and washed first with alcohol and then with ether. It is then dried at 100°–110° and weighed. Care should be taken in weighing as the compound is somewhat hygroscopic.

The filtrate from the digitonin-cholesteride is concentrated and after adding water is shaken out with petroleum ether or ether. The excess of digitonin remains in the aqueous alcoholic solution, whereas cholesterol esters, fats and other lipoids dissolve in the ethers. The petroleum or ether solution is divided into 2 parts, one serving for the isolation of the esters and the other for their quantitative estimation. For the latter purpose the petroleum or ether is distilled off, and the residue saponified by warming with alcoholic potassium hydroxide. The cholesterol set free is then shaken out with petroleum ether and estimated as above. This second precipitate gives the amount of combined cholesterol which was originally present as ester.

The writer's mode of procedure,[3] which was worked out before the appearance of Windaus' later paper, differs slightly in detail from that recommended by him.

[1] *Ber.*, 1909, **42**, 238.
[2] *Zeit. physiol. Chemie*, 1910, **65**, 110.
[3] Fraser and Gardner, *Proc. Roy. Soc.*, 1910, B, **82**, 560.

After precipitating the alcoholic solution of the extract with a slight excess of digitonin in 95% alcohol, the mixture after standing some hours is evaporated to dryness in a vacuum desiccator. The precipitate is then washed by decantation with ether into a previously tared filter paper until the ethereal washings give no residue on evaporation. Care should be taken to use the minimum volume of ether possible. The excess of digitonin is then washed away by warm water. In most cases the filtration is tedious and it was often found more satisfactory to use a tared paper rather than a Gooch crucible, care being taken to subject the tare to exactly the same treatment as the filter paper which received the precipitate. The washing with water is continued until there is no residue on evaporation, or until the washings cease to froth on shaking. The precipitate is then dried in an air-oven at 110° and weighed in a stoppered bottle. In order to estimate the esters the ethereal washings containing the fat and esters may be saponified with sodium ethoxide in the manner described below. It was found preferable, however, when the amount of material available was sufficient, to divide the original extract into two halves. In one-half the free cholesterol is estimated as above and the other half is saponified and the total free and combined cholesterol again estimated. To saponify the esters the extract is dissolved in ether and a large excess of an alcoholic solution of sodium ethoxide added. The saponification of the esters is always complete on 24 hours' standing in the cold. The precipitated soaps are filtered and well washed with ether. The filtrate containing the total cholesterol is thoroughly washed by repeated shaking in a separator with water. The ethereal solution thus obtained is evaporated and the cholesterol estimated as above. Should it happen that the quantity of soap produced is large, it is necessary to allow the ether adherent to evaporate, grind the soap up with excess of salt and extract in a Soxhlet with ether. With small quantities of soap this is unnecessary.

The weight of digitonin-cholesteride $\times$ 0.243 gives the weight of cholesterol. For most purposes it is sufficient to take $\frac{1}{4}$ the weight of the compound.

Both the methods of procedure described have given excellent results in the writer's laboratory. The digitonin method has been adversely criticised by various writers. It has been pointed out that errors are introduced owing to the slight solubility of the compound in ether or petroleum ether, and that this solubility may be increased if the ether already contains fat or other lipoid substances. When a fair quantity of the compound is weighed such errors are negligible, but become more serious as the quantity dealt with becomes smaller. Such errors are, however, inherent in every gravimetric method of analysis when the quantities to be estimated fall below a certain limit. When the quantity of cholesterol to be estimated is very small the writer measures the volume of ether used and makes a correction for the compound dissolved. It is better to make this correction by means of a control experiment with digitonin-cholesteride, keeping the conditions as similar as possible to those in the actual estimation. Similar insoluble digitonin

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compounds are given by some other members of the sterol group, *e.g.*, bihydro-cholesterol, coprosterol, some phytosterols. Ψ-coprosterol and the non-crystalline sterol alcohols of fæces are not precipitated.

The comparative behaviour of the different members of the phytosterol group has not as yet been investigated.

Method of Adrien Grigaut.[1]—Grigaut employs a colorimetric method depending on Liebermann's reaction, viz., when cholesterol is dissolved in acetic anhydride and to the cold solution a drop of strong sulphuric acid is added, a succession of colours—red, blue, bluish-green and finally green—gradually appear. His mode of procedure in case of blood-serum is as follows: 2 c.c. of serum are placed in a small tap funnel with graduation marks at 15 c.c. and 30 c.c., alcoholic sodium hydroxide (1 in 200) are then added up to the 15 c.c. mark and finally ether to the 30 c.c. mark. The funnel is now stoppered and inverted several times to mix the contents thoroughly. After standing until the ethereal layer separates, the aqueous lower layer is run off and the ethereal solution washed twice by shaking each time with 20 c.c. of water. After draining off the wash water the ethereal solution is transferred to a porcelain dish and evaporated to dryness. The fatty residue is dissolved in 5 c.c. of chloroform and transferred to a graduated test-tube of 10 c.c. capacity. To this is now added 2 c.c. of pure acetic anhydride and 3 drops of a solution of sulphuric acid of 66° Bé. At the same time he introduces into a similar graduated tube 5 c.c. of a standard chloroform solution of cholesterol (containing 0.06 grm. per 100), 2 c.c. of acetic anhydride and 3 drops of acid. The tubes are allowed to stand for half an hour for the colour change to become stationary (green). 5 c.c. of the two coloured solutions are poured into the two tubes of a colorimeter, and the one with the deeper tint diluted with a mixture of chloroform, acetic anhydride and sulphuric acid in the above proportions until the tints in the two tubes are equal. If then *n* is the number of c.c. of the diluted solution, the amount of cholesterol *P* contained in a litre of serum is given by the following formula:

(1) In the case in which the solution to be estimated is diluted

$$P = 0.30 \times n \text{ grm.}$$

(2) In the case in which the standard solution is diluted

$$P = \frac{7.50}{n} \text{ grm.}$$

In order to estimate the cholesterol in a solid tissue, 0.2 to 1 grm. of the tissue, according to its cholesterol content, is put into a 90 c.c. flask with 30 c.c. of alcoholic sodium hydroxide (1 in 100) and heated on a water-bath until the tissue is dissolved and the volume of the mixture reduced to 15 c.c. The 15 c.c. of liquid are then introduced into the tap funnel described above and

[1] *La cycle de la Cholestérinémie*, p. 28.

the flask washed with 15 c.c. of ether which is also added. The subsequent procedure is exactly the same as in the case of serum.

The weight P of cholesterol contained in 1 kilo of tissue will be obtained by the preceding formula in which the variable weight p of the tissue taken is introduced.

(1) In case in which the solution to be estimated is diluted

$$P = \frac{0.6\, n}{p}\ \text{grm.}$$

(2) In case in which the contents of the tube containing standard solution are diluted

$$P = \frac{15}{n \times p}\ \text{grm.}$$

The method is open to the serious objection that the Liebermann reaction is a progressive one and the time taken to arrive at a given tint will depend on the amount of cholesterol present. Experience, however, shows that when the percentages of cholesterol do not differ very markedly, as for instance in different sera, good comparative results are obtained. Owing to the small quantities of material taken the error must be considerable when the units are multiplied up to 1,000 c.c. or 1 kilo.

The method gives only the total cholesterol and does not permit of differentiation between free cholesterol and its esters. The most serious objection to the method is that the Burchard-Liebermann reaction is given by other substances than cholesterol, among which may be mentioned cholesterol esters, hydroxycholesterol, coprosterol, etc., and the reagent gives coloured solutions with resins and other substances not belonging to the sterol group.

Leonhard Wacker[1] has moreover recently shown that human fat contains in addition to cholesterol, another substance of the sterol group, which is obtained along with the cholesterol in the unsaponifiable matter. This substance constitutes a very considerable part of the lipoid matter dissolved in fat. It is a wax-like compound, melting at $25°-32°$, and gives colour changes with the Liebermann reagent, but is not precipitated by digitonin.

The writer has also recently isolated similar substances from human fæces. They are readily obtained from the unsaponifiable matter of fæces, after the bulk of the coprosterol has been removed by crystallisation from acetone and the last traces precipitated as digitonin compound, by distilling in superheated steam and subsequently fractionating in a high vacuum. They give a reaction with Liebermann's reagent, but are not precipitated by digitonin.

Though the colorimetric method gives useful results with sera, results by this method are of little value in the case of solid tissues.

<center>ERRATA IN VOL. II.</center>

Page 484, line 11 for "incholesteryl" read "isocholesteryl."
Page 487 line 7 from bottom, for "$(\alpha)_D$" read "$[\alpha]_D$."

[1] *Zeit. f. physiol. Chemie,* 1912, **80,** 404.

WOOL, GREASE AND CLOTH OILS.

By AUGUSTUS H. GILL.

The nature of the hydrocarbons obtained from distilled wool-grease (Vol. II, p. 503) has been investigated by Gill and Forrest.[1] They were found to be olefines boiling at 110° to 193° under 1 mm. pressure and having formulæ corresponding to $C_{20}H_{40}$ (eicosylene) to $C_{30}H_{60}$ (triacontylene). They were white crystalline substances resembling paraffin; some oily lower boiling compounds were also observed.

ERRATA IN VOL. II, INDEX.

Page 515, for "Amidol" read "Arnidiol."
Page 515, for "Caratol read "Carotol."
Page 516, for "Electric Conduct" read "Electric Conductivity," "Singili" read "Gingili," "Hydrocaratol" read "Hydrocarotol."

[1] *J. Amer. Chem. Soc.*, 1910, 32, 1071.

HYDROCARBONS

By R. LESSING, Ph.D.

Hydrocarbons occur in nature ready formed in natural gas, petroleum oil, earth-wax, shale and coal, or are obtained from these and a great number of vegetable substances by ordinary or destructive distillation. The fact that these natural products as well as the distillates obtained from them consist invariably of mixtures, mostly of a highly complex character, renders the identification and estimation of individual compounds exceedingly difficult, and in many cases practically impossible. The analyst is therefore confronted in many cases with problems which have so far not been solved satisfactorily.

Physical Methods of Separation.

Fractional distillation offers a means of separating groups possessing similar physical characteristics within certain narrow limits. But even where hydrocarbons of one series, such as paraffin or benzene derivatives are concerned, their quantitative isolation is a very lengthy and tedious operation involving many fractionations on account of the close proximity of the boiling points and the consequent overlapping of fractions. The problem becomes, however, still more complex when hydrocarbons of different series are present, and inseparable mixtures of constant boiling point are formed. Thus, the addition of aliphatic to aromatic hydrocarbons exerts a depressing influence and a small addition of n-hexane may lower the boiling point of benzene to 65°.[1]

A further difficulty is introduced by the instability of the higher members at the temperature at which they boil; distillation is then accompanied by decomposition or molecular changes ("cracking") and must be carried out at reduced pressure or with the aid of superheated steam.

Notwithstanding these limitations, distillation is an indispensable method in examining hydrocarbon mixtures, both for the determination of the boiling range without fractionation of the distillates and for the separation of fractions boiling within more or less narrow limits. The latter operation, by which such complex mixtures as crude petroleum oil or coal tar are resolved into groups of comparative simplicity, almost invariably precedes the application of specific chemical reagents.[2]

[1] Jackson and Young, *Trans.*, 1898, 73, 922.
[2] For a closer study of the subject see Sydney Young, *"Fractional Distillation,"* London, 1903.

The range and number of fractions to be taken depends on the scope of the examination and may vary between wide limits. The sp. gr. of each fraction is determined, and often the refractive index, optical rotation, viscosity, flash point, etc., will give useful information.[1]

Fractional distillation—at low temperatures—can also be applied to hydrocarbons which are gaseous at ordinary temperature and pressure. Lebeau and Damiens[2] cool mixtures of the lowest members of the paraffin series and hydrogen, to the temperature of liquid air, where methane has still a tension of 80 mm. of mercury whilst the higher members are liquid. The gaseous portion is separated, measured and analysed and the condensate is successively fractionated from baths of solid carbon dioxide and acetone, and petroleum ether cooled by liquid air, yielding binary mixtures of ethane and propane, propane and *iso*-butane, the components of which can be estimated by combustion.

An apparatus has been devised by which the constituents of such complicated mixtures as coal gas can be accurately determined in a practical way, largely by the aid of fractional distillation.[3]

Amongst other physcial methods of separating hydrocarbons, filtration or diffusion through Fuller's earth, bone charcoal and other porous materials, has little importance from the point of view, of general analysis but "cold fractionation" by solution or precipitation is of value in certain cases. S. Aisinmann[4] studied the solubility of Russian petroleum distillates and residues in alcohol. Alcohol is added from a burette to 10 c.c. of the oil at ordinary temperature, until complete solution takes place. Solubility decreases with a rise in boiling point. With heavy oils it is necessary to shake with an excess of alcohol and decant the alcohol solution repeatedly until an insoluble residue remains behind. Hydrocarbons rich in carbon require more alcohol for solution, but dissolve, on the other hand, less alcohol, than those poorer in carbon, a fact conducive to good separation.

Fractional precipitation can be effected according to Charitschkoff[5] by

[1] A distillation method for the estimation of toluene in commercial toluol, solvent naphtha, and other coal-tar distillates has been devised by H. G. Colman (*J. Gas Lighting*, 1915, 129, 196, 314) while this article was in the press.
  The principle of the method is to estimate the volume of the portions boiling below 105° and above 117°, by which figures the toluol percentage can be ascertained from an empirically constructed table. It is necessary to prepare the sample by a careful distillation up to 145° using a Young 12-bulb "pear" fractionating column, and to add to the distillate definite quantities of pure benzene, toluene, and xylene in order to bring the expected percentage of toluene within the range of from 50 % to 75 % for which only the table is correct. Corrections for paraffin contents are made by ascertaining the sp. gr. of the fraction 105° to 117° and deducting 0.75 % toluol for every 0.001 that the sp. gr. is found below 0.868 from the total toluol contents of the mixture.
  Another method for the same purpose but only applicable to comparatively pure toluols has been proposed by D. Northhall-Laurie (*Analyst*, 1915, 40, 384). He distils 200 c.c. of the sample at a uniform rate collecting the first quarter as distillate and leaving the last quarter in the distilling flask. The boiling points of these two fractions are ascertained in a special apparatus and the results plotted on a graph, from which the percentage can be read off directly. The graph is also available for estimating benzene and xylene, in commercial toluols.
  For both methods it is necessary to consult the original publications as they largely depend for their accuracy on the strict observance of manipulative details, as well as on the empirical tables.
  [2] *Compt. rend.*, 1913, 156, 144, 325.
  [3] See also G. A. Burell and J. W. Robertson, *J. Ind. Eng. Chem.*, 1915, 7, 17, 112.
  [4] *Dingl. pol. J.*, 1895, 297, 44.
  [5] *Chem. Zeit.*, 1904, 287, 87.

dissolving petroleum oils in amyl alcohol and separating the fractions successively by the addition of ethyl alcohol in small portions.

For the removal of oxygen- and sulphur-containing asphaltic compounds from hydrocarbon oils Holde's[1] method is widely used. From 5 to 20 grm. oil are well shaken in a clear glass bottle with 40 volumes of "Normal" benzin (sp. gr. at 15° C. 0.695–0.705; boiling from 65° to 95° C.; free from unsaturated and benzene hydrocarbons). After standing for 24 hours at a temperature of from 15° to 20° C. protected from direct sunlight, the solution is filtered through a double filter and the residue washed with the same benzin until a drop of the filtrate leaves no oily residue on evaporation. The benzin solution then contains only the hydrocarbon oil. The asphaltic residue may be dissolved in coal tar benzene and, after evaporation of the latter, weighed (compare Vol. III, p. 54).

By this method only the hard asphalt is removed. Soft asphalts can be separated by dissolving the oil in 25 volumes of ethyl ether and adding to the solution, drop by drop and with continual shaking, 12.5 volumes of 96% (by weight) alcohol. After 5 hours' standing the precipitate is filtered and washed with a mixture of ether and alcohol in the same proportions (1 : 2) until 20 c.c. of the filtrate leave on evaporation no oily residue but only traces of a pitchy substance. The precipitate is dissolved in benzene and, after evaporation of the latter, weighed. If it is light in colour and likely to contain paraffin wax, this is separated by treatment with absolute alcohol in an extracting apparatus, preferably after mixing it with sand or alcohol extracted charcoal.

F. Schwarz[2] uses butanone (methyl-ethyl-ketone) which has been saturated with water, in which hydrocarbon oils are soluble and asphaltic bodies insoluble, in the place of either of the two preceding reagents, thereby obtaining hard and brittle asphalt residues (for details see page 246).

The estimation of solid paraffin in a petroleum distillate (boiling above 300° C.) is also based on a method of fractional solution. 5–10 grm. of an oil, or 0.5–1.0 grm. of a solid paraffin are dissolved at room temperature in a mixture of equal parts of ethyl ether and absolute alcohol; the temperature is then lowered to −20° C. and more ether-alcohol added so that all oily portions are kept in solution and only flakes of paraffin remain suspended. Oils containing much paraffin should be first dissolved in ether, the alcohol being added subsequently. The paraffin is filtered by suction on a funnel surrounded by a cooling mixture, washed with cooled ether-alcohol and then dissolved with hot benzene and, after evaporating the solvent and drying at 105° C., weighed. In view of the not inappreciable solubility of paraffin in the ether-alcohol mixture, a correction is made by adding to the result 0.2% for perfectly clear oils, 0.4% for semi-solid masses, and 1.0% for solid paraffins.

A method which must be classed amongst methods of fractional solution,

[1] *Unters. der Kohlenwasserstoffōle und Fette*, Berlin, 1913, page 42.
[2] *Chem. Zeit.*, 1911, 35, 1417.

has been devised by Eldeleanu[1] who extracts aromatic and other unsaturated hydrocarbons from petroleum distillates by means of liquid sulphur dioxide at low temperature. The process is practised as a works operation on the large scale for refining lamp oils, but can also be used in the laboratory. If a petroleum distillate is mixed with liquid sulphur dioxide this is at first dissolved, but later 2 layers are formed, the lower one being a solution of the hydrocarbons of high carbon content in sulphur dioxide, whilst some of the gas is dissolved in the upper layer of paraffins or naphthenes. A special burette is used having stopcocks at both ends which by clips are prevented from being forced out. The oil to be tested is cooled in the burette to $-10°$ C., liquid sulphur dioxide is then added (which by its own evaporation cools itself to the same temperature) until the 2 layers are just forming. One-third of the volume of the lower is added in excess, the burette is then shaken and allowed to stand until separation is complete. The extract is run off, and the operation repeated twice with more sulphur dioxide. The bulk of the gas is allowed to evaporate in the air from both fractions and these are finally washed with water. No appreciable chemical action of the sulphur dioxide on the hydrocarbons takes place, but sulphur compounds are acted upon.

Chemical Methods.—The difficulties attached to the physical methods of separation apply almost in the same degree to the chemical treatment of hydrocarbon mixtures. Most specific reagents do not enable the analyst to identify or isolate individual compounds but act only as group reagents. There is also, as in the case of the physical examination, always a tendency of different groups overlapping. For instance, concentrated sulphuric acid which is frequently used to remove unsaturated compounds from saturated hydrocarbons is—contrary to statements in most text-books—capable of attacking paraffin hydrocarbons on prolonged contact and particularly when containing some sulphuric anhydride; a nitrating mixture, the typical reagent for aromatic hydrocarbons, will also attack naphthenes,[2] and similar examples can be found with nearly all the usual reagents.

It is important to remember that nearly all commercial hydrocarbon products, unless highly refined, contain members of different series; thus petroleum oils from practically any source contain besides aliphatic or naphthene (polymethylene) hydrocarbons, benzene homologues sometimes up to 40%;[3] on the other hand coal tar, especially in its lower fractions, comprises a number of aliphatic hydrocarbons. Shale oil distillates are mixtures of chain and cyclic compounds, and spirit from "cracked" oils which is likely to attain commercial importance in the near future, comprises a variety of hydrocarbons of varying degree of saturation.

Although the fact of hydrocarbons of different types frequently appearing

[1] Engler und Ubbelohde, *Zeit. angew. Chem.*, 1913, 26, I, 177.
[2] According to Worstall (*Amer. Chem. J.*, 1898, 20, 202) normal hexane yields some of the primary nitro-compound when boiled with nitric acid during a long period.
[3] Jones and Wootton, *J. Chem. Soc.*, 1907, 91, 1146; J. Steuart, *J. Soc. Chem. Ind.*, 1900, 19, 986.

together seems to justify a *prima facie* case for a systematised series of tests, no such scheme of general applicability has ever been devised. The kind of reagents employed and the sequence of operations must vary according to circumstances. Generally it will be found useful to proceed in this order: unsaturated aliphatic (olefines, acetylenes) and partially saturated hydro-aromatic compounds, aromatic compounds, naphthenes, paraffins. In most cases commercial requirements will not entail a complete examination of a hydrocarbon mixture, but only definite tests for certain properties or components will be specified.

Olefines.—The estimation of olefines by means of their bromine absorption, by which at any rate comparable values can be obtained, has been dealt with at length under this heading in Vol. III.

In order to distinguish between added and substituted bromine which will both be covered by the bromine absorption of, say, a mixture of olefines and aromatic hydrocarbons, Parker C. McIlhiney[1] estimates the hydrogen bromide formed in the latter case.

The sample is dissolved in 10 c.c. of carbon tetrachloride in a glass-stoppered bottle and 20 c.c. of $N/3$-bromine solution in the same solvent are added. After a few minutes 20 to 30 c.c. of a 10% solution of potassium iodide are added, care being taken that no bromine is lost; if necessary the mixture must be cooled and the stopper and neck of the bottle wetted with potassium iodide solution. The bottle is then shaken to ensure the absorption of the bromine and hydrogen bromide by the aqueous solution. The iodine present is now titrated with $N/10$-sodium thiosulphate. By then adding 5 c.c. of a neutral 2% solution of potassium iodate, a quantity of iodine equivalent to the hydrogen bromide formed is liberated and on titrating this, the bromine solution figure may be calculated. All solutions should be tested for acidity and a blank test made. Whilst addition of bromine is instantaneous, the amount of substitution is somewhat affected by the time of contact.

The bromine absorption may not only serve for the estimation but also for the removal of unsaturated from saturated hydrocarbons, which can be separated by distillation from the higher-boiling bromo-compounds.

Sometimes the iodine number is determined in preference to the bromine absorption. For this purpose the employment of Wijs' method is advisable and preferable to that of Hübl. In the case of crude distillate it is necessary to remove any hydrogen sulphide that may be present by shaking with a solution of lead acetate.[2]

A simple method of estimating unsaturated aliphatic or hydro-aromatic hydrocarbons in general consists in shaking the mixture repeatedly in a burette with 10 to 30% by volume of concentrated or fuming sulphuric acid until the volume shows no further reduction. The loss of volume indicates the

[1] *J. Amer. Chem. Soc.*, 1899, 21, 1084.
[2] Graefe, *Zeit. angew. Chem.*, 1905, 18, 1580.

percentage of unsaturated compounds. Heating must be avoided and if aromatic or naphthene hydrocarbons are present, the acid must be added very carefully to avoid a violent action, as these are sulphonated at high temperature by concentrated, and even at slightly raised temperature by fuming sulphuric acid.

A qualitative test for olefines by means of mercuric acetate has been devised by Balbiano and Paolini.[1] 3 to 4 c.c. of a hydrocarbon mixture are shaken with 10 c.c. of a saturated solution of this salt. If olefines are present a deposit of white crystals will be observed after 24 hours.

Tausz[2] based a method for the estimation of olefines on this behaviour. 50 c.c. of the hydrocarbon mixture are shaken with 150 c.c. of mercuric acetate solution for 5 minutes and heated with it for 3 hours under a reflux; the product is then steam distilled, all operations carried out in the same, specially designed flask. The distillate is washed with dilute sodium hydroxide solution and then with sodium hydrogen sulphite solution to remove acetic acid, aldehydes and ketones respectively. The remaining portions are the saturated hydrocarbons. The unsaturated portion can be recovered by decomposing the complex mercury salts with dilute hydrochloric acid.

For the estimation of gaseous olefines, Lebeau and Damiens[3] proposed the use of a 1% solution of vanadium pentoxide in concentrated sulphuric acid or of a 6% solution of uranyl sulphate in the same acid. Either of these solutions will take up 150 times its volume of ethylene very rapidly.

The formation of complex mercury salts is also used by these authors to estimate acetylene. A solution containing 25 grm. of mercuric iodide and 30 grm. of potassium iodide in 100 c.c. of water is capable of absorbing 20 times its volume of acetylene. On making the solution alkaline, a white precipitate of the complex salt is formed. They claim that olefines do not react and can be separated from acetylene by this method, but this appears to be doubtful in view of the above-described action of mercuric acetate on olefines.

Aromatic Hydrocarbons.—In view of the commercial importance of coal-tar derivatives, the hydrocarbons of the benzene series have been studied more closely than others, and partly for that reason and partly on account of their properties it is less difficult to isolate and identify individual members of the series.

Sulphonation of aromatic hydrocarbons by heating them with concentrated or fuming sulphuric acid has been mentioned above. From the sulphonic acids thus produced and separated from mixtures, the hydrocarbons may be recovered by distillation in superheated steam.

A more general and useful method by which aromatic hydrocarbons may be

[1] *Chem. Zeit.*, 1901, 25, 932. *Ber.*, 1902, 35, 2994; see also K. A. Hofmann and J. Sand, *Ber.*, 1900, 33 1340, 1353; 1901, 34, 2906; Denigès, *Bull. Soc. chim.*, 1898 [3], 19, 494.
[2] Dissertation, Karlsruhe, 1912.
[3] *Compt. rend.*, 1913, 156, 557.

recognised and isolated from mixtures is nitration. The carefully fractionated hydrocarbons are treated with 2 to 5 times their volume of a nitrating mixture consisting of 1 volume nitric acid (sp. gr. 1.45–1.50) and 1.5 to 2 volumes of sulphuric acid (sp. gr. 1.85). With good stirring or shaking, careful mixing and low temperature, mostly mono- and di-nitro-products are formed. At higher temperature or with great excess of acid, tri-nitro-compounds will be formed.

When no further action takes place, the nitration is completed and the mixture separates into 3 layers. The acids form the bottom layer, the nitro-compounds are in the dark-brown middle layer, and the unattacked hydro-carbons in the upper layer. The two upper layers are soluble in each other to a certain extent. The acids are either separated first or after dilution with water. The hydrocarbons and nitro-products are washed with water and a little sodium carbonate solution, and are separated by distillation with or without steam. The nitro-compounds, if solid, can be identified by their melting points, or else by reduction 'to their amines and possibly the conversion of the latter into acyl derivatives or salts.

Formaldehyde, which although of fairly general applicability is not yet being employed to the extent which it deserves in hydrocarbon analysis, was suggested for this purpose by A. Nastjukoff in 1904.[1] He found that a mixture of concentrated sulphuric acid and formaldehyde solution reacts with all unsaturated cyclic hydrocarbons forming insoluble compounds, termed "formolite." Neither saturated hydrocarbons of any kind nor unsaturated chain hydrocarbons show this behaviour, but all aromatic and partially saturated hydro-aromatic hydrocarbons, as well as terpenes and hydroterpenes, yield "formolites."

The sample is mixed with its own volume of concentrated sulphuric acid and half its volume of a 40% formaldehyde solution is then slowly added, the mixture being well cooled the whole time. (If the yield of "formolite" is higher than 50%, the ratio of sample, sulphuric acid and formaldehyde to be taken is 1 : 2 : 1.) It is then shaken until the temperature no longer rises. In many cases, particularly with viscous oils, it is advisable to dilute the sample previously with light petroleum spirit free from "formolite" yielding hydrocarbons. After half an hour's standing, the mixture is poured into ice water, the flask being washed out with water. An excess of ammonia is added to the acid solution and the precipitate is filtered and washed on a vacuum filter. The precipitate from heavy oils is first washed with petroleum spirit to remove unattacked oil, then with water until free from ammonia and is then dried at 105° C. In view of the difficulty of filtration and washing, it is sometimes necessary to repeat the extraction with spirit and water of the dried and powdered precipitate.

Nastjukoff calls the number of grams of precipitate per 100 c.c. of original

[1] *J. Russ. Chem. Phys. Soc.*, 1904, 36, 881; 1910, 42, 1596.

oil the "formolite number" of the oil or hydrocarbon mixture. The precipitates are of yellow to brown colour and practically insoluble in the usual solvents except to a small extent in chloroform.

The composition of the "formolites" is not yet cleared up; the unsaturated cyclic hydrocarbons combined with formaldehyde are equal to about 80% of the "formolite." V. F. Herr[1] proposed the use of methylal in place of formaldehyde on account of its solvent power for oils.

Naphthalene is now universally estimated by the picrate method. Some useful results were obtained by W. P. Jorissen and J. Rutten[2] in an investigation on the conditions of solubility of picric acid, naphthalene and naphthalene picrate. They found on the basis of the phase rule that for a complete conversion of the hydrocarbon into its picrate, it is necessary that the aqueous solution of picric acid employed should be supersaturated and in fact a certain amount of crystals should be present as a bottom body. A solution made by adding 100 c.c. of water to 2.7 grm. of pure picric acid will fulfil this condition. The usual alkalimetric estimation of the picric acid excess, by $N/10$ sodium hydroxide, may be replaced by an iodometric method. 25 c.c. of a solution of potassium iodide and iodate (150 grm. KI, 30 grm. KIO_3, 400 c.c. H_2O) are added to 100 c.c. of the picric acid solution and are titrated with $N/10$ thiosulphate solution and starch as indicator. The colour change is then more distinct than that of the phenolphthaleïn in the alkali titration[3] (see also page 266).

Naphthenes (Polymethylenes).—After removal of the unsaturated aliphatic and the aromatic hydrocarbons from a mixture, the polymethylenes and paraffins remain. Their separation is particularly difficult on account of the similarity of their physical and chemical characteristics. Specific gravity and refractivity will give some indication for their differentiation; generally qualitative tests will meet the case.

PHYSICAL PROPERTIES OF THE SIMPLEST CYCLOPARAFFINS.[4]

Name	M. p.	B. p.	d_4°	n
Cyclopropane	−126.0°	about −35°	....	....
Cyclobutane	liquid at −80°	11.0–12.0°	0.7038	1.37520
Cyclopentane	liquid at −80°	49°	0.7635	1.40855
Cyclohexane	+ 6.4°	81°	0.7934	1.4266
Cycloheptane	....	117.0–117.5°	0.8252	1.44521
Cyclooctane	+ 11.5°	145.3–148.0°	0.850	1.45777
Cyclononane	....	170.0–172.0°	0.785	1.4328

Hydrocarbons of the polymethylene series are not attacked by a mixture of sulphuric and nitric acids at the ordinary temperature, nor readily on moderate heating.[5] With fuming nitric acid the behaviour of pentamethylene

[1] *Chem. Zeit.,* 1910, **34,** 893. See also Severin, *Chem. Zeit.,* 1910, **34,** 840.
[2] *J. Soc. Chem. Ind.,* 1909, **28,** 1179.
[3] E. Schlumberger, *J. f. Gasbel.,* 1912, **55,** 1260.
[4] R. Willstätter and J. Bruce, *Ber.,* 1907 **40,** 3981.
[5] Francis and Young, *Trans.,* 1898, **73,** 928.

and hexamethylene differs widely from that of their methyl derivatives, for the former are only attacked when heated and yield chiefly the corresponding dibasic acids—glutaric and adipic acids—whilst the methyl derivatives and especially methylpentamethylene, are acted on rapidly at the ordinary temperature with evolution of heat and for the most part broken down.

If heated with dilute nitric acid in sealed tubes, the polymethylenes yield according to Konowaloff[1] mono-nitro-compounds which are of tertiary character if the original hydrocarbon has a side chain. 5 c.c. of the hydrocarbon are heated in a sealed tube with 25 c.c. of nitric acid of sp. gr. 1.025 to 1.075 for 12 hours at 120° to 130°. The product is washed with sodium carbonate solution and water and dried with calcium chloride. The unattacked hydrocarbon is distilled off. If the distillation is carried out under reduced pressure, it can be continued until the nitro-compounds are carried over. Secondary nitro-compounds are separated from the tertiary by extraction of the mixture with alcoholic or dilute aqueous potassium hydroxide.

Polymethylenes may be qualitatively tested by de-hydrogenating them according to Sabatier's method. Cyclohexanes are converted into aromatic hydrocarbons by passing them over finely divided and freshly reduced nickel at 300° C. and can then be easily identified by the formol reaction.

Zelinsky[2] converted *cyclo*hexane quantitatively into benzene and hydrogen by passing it 3 times very slowly over palladium black at 300° C.

In this connection mention may be made of S. Fokin's[3] proposal to estimate the degree of unsaturation of organic compounds by the determination of the "hydrogen number," *i.e.*, the number of c.c. of hydrogen (at 0° C. and 760 mm.) which are absorbed by 1 grm. of the substance when submitted to hydrogenation. A distillation flask (50 to 150 c.c.) having a small beaker fused inside on the bottom is connected by means of the side tube to a gas burette and a gas holder containing hydrogen. In the small beaker are placed 0.1 to 0.2 grm. of molecular platinum moistened with 0.25 to 0.5 c.c. of water, and in the flask the substance to be examined and 20 to 30 c.c. of alcohol free from dissolved oxygen. The flask is shaken by a shaking machine and the hydrogen absorbed is read off when constancy is obtained.

Willstätter[4] had previously hydrogenated polymethylenes by means of Sabatier and Senderens' method.

A method for the estimation of the total hydrogen contained in a substance is due to A. P. Lidoff.[5] From 0.25 to 0.5 grm. of the substance is mixed with about 1 grm. of powdered magnesium, which has been ignited previously in a current of hydrogen and the mixture is introduced into a thick-walled test-tube made out of combustion tubing. This tube should

[1] *Ber.*, 1895, 28, 1863., *Chem. Zentr.*, 1898, i, 926; 1899, i, 966; 1902, i, 564.
[2] *Ber.*, 1911, 44, 3121.
[3] *Russ. Phys. Chem. Soc.*, 1908, 40, 700.
[4] *Loc. cit.*
[5] *J. Russ. Phys. Chem. Soc.*, 1907, 39, 195, 208. *Zeitsch. anal. Chem.*, 1907, 46, 357.

be 130 mm. long and 9 mm. in diameter; a layer of magnesium powder is placed above the mixture so as to fill the tube to a height of about 80 mm. After connecting the open end of the tube with a gas measuring burette, the layer of magnesium is heated to redness and the heat is then gradually extended to the lower part of the tube containing the mixture. The usual precautions are taken in adjusting the zero of the burette and in reading the volume of the hydrogen.

The method has been applied by the author to the examination of hydrocarbon oils.

Paraffins.—The paraffins possessing the smallest reactivity remain behind after removal of all the other hydrocarbons. They are, however, liable to be attacked by strong fuming sulphuric acid on prolonged heating. Whilst practically unattacked by nitric acid in any form at ordinary temperature, the normal members may be slowly decomposed by fuming nitric acid, when heated on the water-bath; but the iso-paraffins are readily attacked under these conditions, a moderate yield of nitro-compounds being obtained.[1]

The estimation of solid paraffin in oils by the ether-alcohol method has been described. It is frequently necessary to estimate conversely the amount of oil in a solid paraffin.

Thus Marcusson[2] bases the identification of a paraffin wax, according to its origin, upon the residual oil which it always contains. 100 grm. paraffin are dissolved in 300 c.c. of ethyl ether and the same volume of ethyl alcohol (96%) is then added; the mixture is cooled in running water and filtered on a Büchner funnel. The filtrate is evaporated again, dissolved in 50 c.c. of ether and precipitated with 50 c.c. of alcohol, cooled to −20° C., filtered and evaporated. The iodine number is determined of the oily residue which may be taken up in benzin for further purification. This is from 3 to 12 with petroleum paraffins and from 18 to 21 with shale paraffins; but similar differences are observed before and after refining with samples of the same origin.

F. Sommer[3] makes use of the formaldehyde method for the same purpose. 20 grm. of paraffin are melted in a flask, 20 c.c. of concentrated sulphuric acid are first added and then gradually 20 c.c. of formaldehyde solution, the flask being shaken and excessive heating avoided. The mixture becomes dark red and is left for 20 minutes on the water-bath; it is then turned into a porcelain basin and left on the water-bath until the paraffin is completely separated. After cooling, the paraffin cake is lifted·off, the acid liquid is diluted with water and the "formolite" is shaken out with chloroform. The chlorofrom solution is evaporated and the residue dried

[1] Francis and Young, *loc. cit.*
[2] *Zeit. angew. Chem.*, 1910, **23**, 1057.
[3] *Petroleum*, 1912, **7**, 409.

at 105° C.   The "formolite" varies from 0.02 to 1.55% and corresponds with the tendency of a paraffin to turn yellow.

F. Epstein and H. Polonyi[1] employ the formation of picrates for the same purpose.   20 grm. of paraffin are melted at low temperature, 0.02 grm. picric acid is added and well stirred in; after allowing the insoluble matter to settle, the paraffin is poured into a mould of tinplate which floats on ice water.   The colour of the solidified cake is compared with a standard sample and 0.05% of oil in paraffin can be estimated.

[1] *Petroleum*, 1912, 7, 594.

BITUMENS.

By S. S. SADTLER.

Bituminous substances, according to Herbert Abraham, are classified as (1) natural and (2) artificial. The natural are bitumens and pyrobitumens. The bitumens are (a) *gaseous*, as natural and marsh gas; (b) *liquid*, as petroleums with paraffin, asphaltic or mixed bases, or malthas ("mineral tars"); (c) *solid*, as mineral waxes like ozokerite (ceresin), montan wax and hatchettite, or mineral pitches like pure, calcareous, siliceous and earthy asphalt(um), or asphaltites like gilsonite, glance pitch, manjak, grahamite. The pyrobitumens are (a) solids occurring fairly pure, such as elaterite (wurtzelite), albertite, impsonite, derived from asphalts, and peat, lignite, bituminous and anthracite coals derived from vegetable growth; (b) solids in which mineral materials predominate, such as bituminous shales and schists. The artificial bituminous substances may be of (1) *animal origin*, as bone tar and bone tar pitch from bones, or fatty acid pitches from animal and vegetable fats, such as stearin pitch (candle tar), cotton-oil-foots pitch, wool-fat pitch, palm-oil pitch, cottonseed-oil pitch; (2) *vegetable origin*, as fatty acid pitches from vegetable oils, resin pitch from saps of *Coniferæ*, pine tar and pine tar pitch from wood and roots of *Coniferæ*, wood tar and wood tar pitch from the hard woods; (3) *mineral origin*, as water gas tar and pitch, sludge pitch, petroleum asphalt (petroleum pitch), blown (oxidized) petroleum asphalt from petroleum; asphalt(um) from malthas, paraffin, etc., from ozokerite, etc., peat tar and pitch from peat, lignite tar (brown coal tar) and pitch from lignite (brown coal), coal tar and pitch, coke oven tar and pitch, blast-furnace tar and pitch, water-gas tar and pitch, generator-gas tar and pitch from bituminous coal; elaterite pitch from elaterite, shale oil and shale oil pitch from bituminous shales.

Asphalt.

Method of Analysis of Total Bitumen in Surface Mixtures.—Weigh 10 grm. in a long tube or deep glass cup with ground stopper and then fill ¾ full with carbon disulphide. Whirl in a centrifuge for several minutes. Decant the solvent into a tared platinum dish and fill the tube ¾ full with fresh carbon disulphide. Stir sediment with a rod and whirl again. This is repeated until the solvent above the sediment remains colourless.

Ignite these extracts in a good draught and burn to an ash. Dry the test-tube with the sediment and weigh. Add correction in the platinum dish and subtract from 100% to obtain the bitumen.

Method of Extracting Bitumens from Binding Courses, Topekas, Etc.—A special extractor (New York Testing Laboratory Type, see Figs. 10 and 11), for analysis of paving mixtures containing broken stone, is used. The bituminous mixture should be warmed until it can be readily broken apart by hand, without fracturing any of the stony particles; 500 grm. of the disintegrated mixture should be packed as tightly as possible in the wire basket and then covered with a disc of cotton or felt of $\frac{1}{4}$ to $\frac{1}{2}$ in. thick; 175 to 200 c.c. of carbon disulphide, carbon tetrachloride, chloroform or
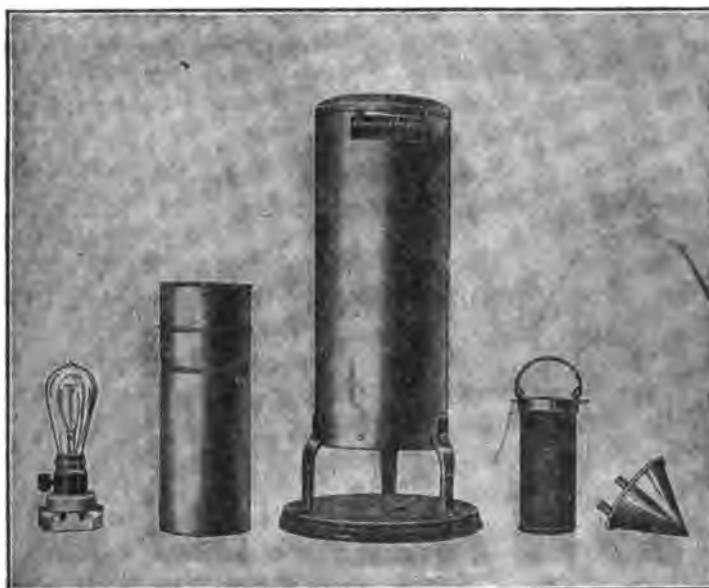
FIG. 10.—N. Y. testing laboratory extractor in parts.

benzol are placed in the inside vessel in which the wire basket is suspended. Cool water should be circulated through the inverted cone condenser which is also the cover of the apparatus and not intended to fit tight. A 16 c.p. carbon filament incandescent lamp is the source of heat. A 500 grm. sample of the mixture should be extracted clean with carbon disulphide in about 3 hours. From 200 to 300 grm. of asphalt block or Topeka type mixture is a sufficiently large sample for that type of material. After extraction, the solvent and matter removed from the sample during the analysis should be burnt to recover any fine mineral particles which may have passed into the extract. The extractors are made entirely of metal.

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Ductility (New York Testing Laboratory Methods).—The sample is melted and poured into brass briquette moulds,[1] the latter first having been amalgamated to prevent the asphalt from adhering. The sample is then cooled and placed in ice water (41° F.) for 15 minutes. Then with a heated knife, the excess of asphalt over 1 cm. thickness is cut off so that the centre and narrowest portion of the briquette is 1 sq. cm. The briquette is placed in water at 77° for 15 minutes, and then drawn out in water at 77° F. in a ductility machine, so regulated that it only moves 5 cm. per minute. The reading on the metre rule when the thread of asphalt breaks is called the ductility of the asphalt.
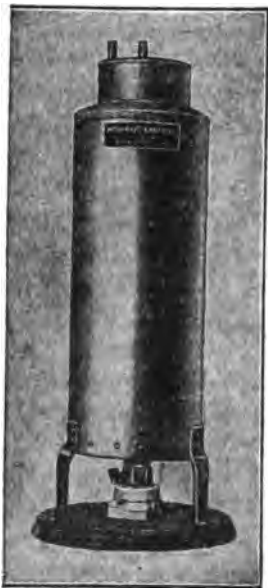
FIG. 11.—N. Y. testing laboratory extractor complete.

Melting-point

Apparatus.—Outer vessel or container for the glycerin bath, a 600 c.c. Jena beaker, Griffin type.

Inner vessel or air-bath 200 c.c. is a lipless Jena beaker.

Chair or support for inner vessel is cut out of $\frac{1}{16}$-in. sheet aluminium.

Cover for inner vessel is cut out of sheet aluminium or brass.

Support for moulds consists of discs of brass with holes tapped for two or four moulds suspended on three hangers.

Commercial glycerin, standard thermometer.

Double thickness 20 mesh iron gauze.

Iron tripod, stand and clamps.

Bunsen or alcohol burner.

Manipulation.—One or more of the brass moulds standing upon a piece of amalgamated brass or tin should be filled with the bitumen under examination. The bitumen may be softened by cautiously heating it in a small casserole or tin box until it is sufficiently fluid to be poured into the mould. After trimming off the upper surface level with the mould, place the sample in water at 77° F. for about 10 minutes. It should then be suspended in the air-bath of the apparatus and the cover and thermometer placed in their proper positions. The temperature of the glycerin bath should also be 77° F. at the beginning of the test.

The apparatus should stand on a double 20-mesh iron gauze, supported on an iron tripod and heated at the rate of 5° F. per minute. The temperature at which the sample of bitumen flows from the mould and first touches the bottom of the inner vessel, is recorded as the melting or flowing point.

[1] Moulds for bitumen and brass collars used in N. Y. T. L. float test apparatus are made by Howard & Morse, Brooklyn, N. Y.

Float Test.—The sample is melted at as low a temperature as possible and then poured into a small brass mould (the same as used in melting point determinations). It is then allowed to cool, placed in water at 41° F. for 10 minutes, the edges then trimmed with a hot knife and cooled for 10 minutes longer. It is then screwed into an aluminium float plate or dish about 3 in. diameter and placed in water at 150° F. starting a stop watch the moment the float is placed in the hot water. The second the water flows through the melted bitumen into the inside of the float mould is taken as the float.

Drip Point.—Melt the sample at as low a temperature as possible and then coat the bulb of a thermometer with the bitumen until a layer $\frac{1}{8}$ to $\frac{1}{16}$ in. thick and about 1 in. long is obtained. Cool and place in an air-bath free from currents, which is heated so that the temperature of the air rises about 2° C. per minute. The temperature on the thermometer when the first drop falls, is noted as the drip point.

Fluxes.[1]

By the term "asphalt content" of a flux or road oil, etc., is meant the residue remaining after heating the oil at 500° F. until the penetration at 77° F. of the residue is 100.

Sp. gr. at 60° F. or 77° F. is determined in a small bottle by weighing in air and water as under Crude Petroleums.

Flash point is determined in a New York State Open Cup Tester. This consists in heating the flux in a 3-ounce tin box with a Fahrenheit thermometer inserted so that the temperature of the flux rises 5° F. every minute. The point at which the escaping gases catch fire or a lighted taper flashes the entire surface of the oil is noted as the flash point.

Loss on 20 Grm. at 212° F.—Weigh out 20 grm. into a 3-ounce tin box and note the exact weight. Then put in an oven at 212° F. and leave for 5 or 7 hours as specifications call for. Take out from oven, cool and weigh. Calculate loss to per cent.

Loss on 50 Grm. at 212° F.—Weigh out 50 grm. and follow procedure as above.

Loss on 20 Grm. at 325° F.—Weigh out 20 grm. in a 3-ounce tin box and put in an electric oven regulated by means of a thermostat at 325° F. for 5 or 7 hours as called for. Calculate loss to percentage as above.

Loss of 50 grm. at 325° F. is estimated in the same way, using 50 grm. instead of 20 grm.

When removing the boxes from the oven it is advisable to tilt them so that on cooling the asphalt is up against one side of the box. This is done when a penetration of the residue at 77° F. is required.

Bitumen soluble in carbon disulphide is estimated as under *Asphalts* (see Vol. III, p. 77).

[1] Methods of the New York Testing Laboratory.

Mineral matter is estimated as under *Asphalts* (see Vol. III, p. 79).

Bitumen Soluble in 88° Naphtha.—Weigh out 1 grm in a 200 c.c. Erlenmeyer flask. Add 100 c.c. naphtha (88° Bé.) and leave over night. Filter through a wide mouth Gooch crucible without suction, wash until the washings are colourless, with 88° naphtha; dry at 212° F. and weigh. If all the asphalt is not washed out of the flask, this is dried and weighed also and the weight of the remaining asphalt added to that on the Gooch. This subtracted from 1 grm. and divided by 100% gives per cent. soluble in 88° naphtha.

Roofing Papers.[1]

Method of Testing.—(1) Make four tests on Mullen (paper) machine. Report average and temperature. (Cut two 3-in. squares, two 6-in. squares, one piece 6×2 in. for subsequent tests.)

(2) Ignite one of the 3-in. squares and calculate per cent. ash.

(3) Extract one 6-in. piece and one 3-in. piece with carbon disulphide in an extractor and calculate per cent. bitumen, etc., as per following formula:

Formula for calculating bitumen, felt and mineral matter in coating.

If A = per cent. ash in original sample.

B = per cent. ash in dry felt.

C = per cent. dry felt in original sample.

A − CB = mineral matter in coating.

100 − C + A − CB = bitumen.

(4) Ignite extracted 3-in. square and calculate per cent. ash in dry felt.

(5) Measure thickness of extracted 6-in. square felt.

(6) Make four tests on Mullen machine for strength of dry (extracted) felt. Report average.

(7) Treat 1 grm. of extracted felt with 100 c.c. of 2% sodium hydroxide in a 200 c.c. Erlenmeyer flask with reflux condenser and boil gently for ½ hour.

Filter in a Gooch crucible, washing with hot water, return fibres to flask and treat once more as above. After washing thoroughly with hot water, dry at 212° F. and weigh. Report per cent. dry felt (wool and hair) removed by 2% sodium hydroxide.

(8) Place 2 in.×6-in. strip in ice water at 41° F. and after 15 minutes work over rods ¾ in., ⅝ in., ½ in., ⅜ in., ¼ in., and 180° flat, reporting the diameter of rod over which roofing cracks.

Weight 3-in. square in grams×16.896 = pounds per 480 sq. ft.

Estimation of Paraffin in Asphaltum.

Richardson's method of purifying the fraction soluble in light benzin by means of concentrated sulphuric acid (Vol. III, p. 82) is claimed

[1] Methods of the N. Y. Testing Laboratory.

by Marck[1] to be tedious and the use of animal charcoal is recommended by him. 1 grm. of asphaltum is dissolved in 2 c.c. of chloroform, and 50 c.c. of light benzin (b. p. 70° C.) are added. After digesting for 1 hour the liquid is filtered through 40–60 grm. of the dry pulverised charcoal resting on cotton wool moistened with petroleum ether, suction being applied. Washing is effected with three to four portions of 30 c.c. of petroleum ether. The bulk of the solvent is distilled off, the remainder expelled in the oven, and paraffin determined in the residue by the method of Engler-Böhm-Holde (solution in ether and precipitation with absolute alcohol at −20° C.).

Separation of Natural and Petroleum Asphalt.—Marcusson has previously recommended the estimation of the oil content as a means of distinguishing between natural and petroleum asphalt, since natural asphalt contains 2–34% oil, practically free from paraffin, while petroleum asphalt contains 26–58% oil, very rich in paraffin. H. Loebell[2] has objected that this method is impracticable in the case of paving asphalts where small amounts of petroleum asphalt are added to Trinidad asphalt as a flux and serve to increase the percentage of oily constituents. Marcusson[3] replies that this fact in itself would be sufficient to show the presence of petroleum asphalt in the natural product and cites numerous examples in support of this contention. Loebell recommends the determination of the paraffin content of the oily portion of the asphalt as a method of distinguishing between natural and petroleum asphalts, the oil from the latter being high in paraffin, but Marcusson points out that certain Roumanian petroleums show a very low paraffin content. The distillation of the asphalts, however, and determination of the acid number of the distillates are of considerable value. Distillates from petroleum asphalts have low acid numbers and free paraffin frequently separates. Natural asphalt distillates have higher acid numbers and show no free paraffin. Determination of the saponification number of the asphalt is also valuable, since this number of natural asphalts runs much higher than that of the petroleum asphalts, 28.5–34 for the former as against 7.6–13.5 for the latter. Of the petroleum asphalts the Californian and Roumanian products have the highest saponification number. Determination of the sulphur content of asphalts is of little value since the sulphur content of certain petroleum asphalts is as high as or higher than that of some natural asphalts.

The Estimation of Natural Asphalt in the Presence of Artificial Asphalt.—Marcusson's method[4] is based on the precipitation of sulphonated bitumens from water solution. 10 grm. of material are shaken with 75 c.c. of an ether solution of hydrochloric acid (prepared by shaking ether with conc. hydrochloric acid, keeping the mixture cold) in three or four portions. After 10 minutes 75 c.c. of water are added and the mixture heated on the steam-bath until no more odour of ether is noticeable. The insoluble matter is filtered

[1] *Zeit. anal. Chem.*, 1913, **52**, 553–556.
[2] *Chem. Zeit.*, **36**, 22–23.
[3] *Chem. Zeit.*, **36**, 801–803.
[4] *Z. angew. Chem.*, **26**, 91–93.

off, dried and extracted with boiling chloroform; the extract is weighed after distilling off the chloroform and drying at 105°. 3 grm. of this bitumen are then treated with 6 c.c. of conc. sulphuric acid and heated on the steam-bath for 45 minutes. The product is treated with 200 c.c. of water. A precipitate settles out which after 1 hour is filtered off, washed free from acid, dried and weighed. This product is the natural asphalt bitumen. The amount of mineral matter in natural asphalt varies enormously, but the valuation of the material is based on its bitumen content. The results are about 4% low. Adding a correction of 4% to results obtained with Trinidad asphalt and coal-tar pitch mixtures, of known composition gave figures fairly close to the theoretical. Petroleum residues react like natural asphalts with sulphuric acid, so that it is necessary to examine the insoluble precipitate for the paraffin content of the oily portion and the acid content of the distillate.

Estimation of the Asphalt Content of Mineral Oils, Petroleum Pitch and Similar Materials.—A series of experiments by Schwarz[1] using butanone instead of acetone as prescribed in the official German methods are here given. Butanone is a good solvent for mineral oil distillates and is used in somewhat the same manner for their separation as acetone; the ready solubility of light mineral oils in butanone renders it advantageous in separating asphalt from dark mineral oils. On treating asphaltic mineral oils at room temperature with the solvent, large amounts of light oil are dissolved, whilst black, very viscous to soft pitchy masses, according to the nature of the material, remain undissolved; a sharp separation of the asphalt from the oil could not be obtained by this treatment, but on heating the butanone to boiling (81°), clear dark solutions were obtained. Butanone has the power of absorbing a significant amount of water, causing a lowering of solvent power and a more perfect separation of asphaltic substances. 1,000 c.c. of butanone at room temperature requires about 110 c.c. of water for saturation; at 20° the water-free material has sp. gr. = 0.805, saturated with water = 0.835. Tests were made on cylinder oils using a butanone-water mixture of sp. gr. 0.835 and of 0.812, the latter dissolving all oils and giving a clean, sharp separation from asphaltic matter. Estimations were made for comparison by using alcohol-ether and normal benzine. Results by the butanone and the alcohol-ether methods with some samples agreed very well, but with others there was a variation due to the nature of the asphaltic matter, soft asphaltic substances being dissolved in greater amount by the latter and hard substances less by the former. With normal benzin, the results showed that with increasing content of butanone-insoluble asphalt, there was increase in the precipitate by benzin, with a darker colouring of the benzin solution; larger amounts of asphalt are precipitated with butanone than with benzin. Experiments were made on solid bitumens (Trinidad asphalt, soft Italian asphalt, Galician). Butanone is superior to the usual

[1] *Chem. Zeit.*, **35**, 1417.

solvents for separating asphaltene from petrolene in that no chemical change is liable to take place in the constituents from overheating, and because the solvent power may be varied by varying additions of water; this makes it possible to separate the asphalt into a series of characteristic constituents. An accurate study of the latter should contribute to the knowledge of the chemical nature of asphalts and the rôle of sulphur in their formation and properties. Water-free butanone is recommended as a substitute for alcohol-ether in the determination in oils; a single precipitation (cooling of the butanone solution in ice-salt mixture and filtration at 15°) separates total paraffin.

Mineral Oils.

Determination of Sp. Gr. of Small Quantities of Mineral and other Oils.[1]—When the quantity is less than 1 c.c. a sp. gr. bottle of 5 c.c. capacity is filled with about 4.5 c.c. of water and weighed. The oil is poured into the bottle on top of the water and the bottle again weighed. Taking d as the sp. gr. of the oil under test, n its volume and f its weight, we have: $d = n/f$. But n is equal to w (wt. of water fully filling the bottle) less e (weight of water and oil) less p_1 (weight of bottle partially filled with water). Thus: $d = (w - e)/(p_2 - p_1)$.

Method of Determining the Sp. Gr. of Heavy Petroleums (Heavy Crude Petroleums, Viscous Lubricants, "Black Oils," etc.).—J. McC. Sanders[2] describes the following method: The apparatus consists of two 100 c.c. burettes, a 100 c.c. cylinder, a thermometer, rubber-tipped glass rod, and a glass tube curved at one end in a semicircle, and having a small rubber bulb fastened on the other. Two mixtures of pure alcohol and water are required, one containing 50% and the other 75% alcohol by volume. The burettes are supported on opposite sides of the cylinder. Into the cylinder about 75 c.c. of the dilute mixture are run from one of the burettes. The oil is poured into the glass tube and the tube is then immersed in the liquid and a small drop of oil is forced out by means of the rubber bulb. If the sp. gr. of the oil is less than that of the mixture of alcohol and water, the drop will rise to the surface and can be removed by touching it with the rubber-tipped rod. In this case a portion of the stronger alcohol is run in from the other burette, well mixed, and another drop of oil forced out. These operations are repeated until the sp. gr. of the mixture and the oil drops are identical. The volume of the two alcohol solutions used to form the mixture are read off from the burettes, and the sp. gr. of the mixture is calculated from the amounts used.

Sulphur (According to Richardson, Private Communication).—About 0.2–0.3 grm. oil (or asphalt) is weighed out on a small cylindrical absorp-

[1] *Petroleum Rev.*, 28, 252.
[2] *Proc. Chem. Soc.*, 27, 250.

tion disc and is burned in an atmosphere of oxygen in a large glass bottle. The oil is kindled by an electric spark which burns a piece of iron wire and the fire is communicated to the disc by a thread of cotton. As soon as the combustion is complete, the bottle is cooled so as to form a vacuum. A solution of sodium dioxide is then drawn in through a separating funnel, and the bottle is shaken so that all sulphur dioxide is absorbed and oxidised by the alkali. The alkali is then poured into a beaker, the bottle rinsed two or three times and the rinsings added to the alkaline solution. This is then boiled, filtered, acidified with hydrochloric acid, taking care to cover the beaker with a watch glass to prevent loss by effervescing. After all the hydrogen dioxide has been boiled out, the solution is neutralised with ammonia, then slightly acidified with hydrochloric acid and the sulphur trioxide precipitated with barium chloride in boiling solution. The mixture is boiled 5 minutes and left overnight. The barium sulphate is collected on a Gooch crucible, dried, ignited and weighed.

Estimation of Sulphur in Petroleum Illuminating and Lubricating Oils.— A large proportion of the total sulphur in many inferior, poorly refined petroleum oils is present as sulphonates or sulphates; these compounds are not included when the sulphur is estimated by ordinary "lamp" methods, since they remain in the wick, and although included when direct oxidation methods are employed, no distinction is made between them and the sulphur compounds normally present in the oil. The method employed by Conradson[1] consists in burning to dryness a weighed quantity of the oil by means of an ordinary kerosene burner and aspirating the products of combustion through two absorption tubes arranged in series and containing a solution of sodium carbonate (6 grm. per litre) previously standardised with $N/10$ acid. 10–20 grm. of illuminating or 5–10 grm. of lubricating oils are usually taken for the determination; the more viscous oils are mixed with an equal volume of highly refined kerosene of known sulphur content, and when the mixture has burned completely, a further 2 c.c. of the kerosene are added and burned to dryness. The sodium carbonate solution (including the rinsings of the apparatus) is either titrated with standard acid or oxidised with bromine and hydrochloric acid and precipitated with barium chloride as usual. The examination of the wick for sulphur compounds is conducted as follows: When only the total sulphur (sulphonates and sulphates) is required, the wick, cut into small pieces, is digested with 0.2 grm. of sodium carbonate and 5 c.c. of concentrated nitric acid until the fibres are disintegrated, when 2 grm. of magnesium nitrate are added and the temperature is gradually raised until the organic matter is destroyed; the sulphur contained in the residue is then estimated by the usual means. When separate estimations are required of the sulphur present as sulphonates and sulphates, the wick is boiled with a solution of barium hydroxide which

[1] *8th Int. Cong. Appl. Chem.*, 1912; Sec. 1, Orig. Comm., 1, 133–136.

is then diluted and filtered. The filtrate, containing the sulphonates, is either oxidised with bromine and hydrochloric acid, or evaporated to dryness with nitric acid (the residue being treated with hydrochloric acid and boiling water), to effect the precipitation of the barium sulphate representing the sulphonates originally present. The sulphur in the residue from the barium hydroxide treatment is estimated by the method employed in estimating the total sulphur in the wick (see *Sulphur Method*, pages 247, 248).

Water in Crude Petroleum.—Weigh out 100 grm. of the thoroughly mixed sample into a 500 c.c. distilling flask. Add 100 c.c. of heavy benzin (62° Bé.) and connect the flask to a water-jacketed condenser. Distil until no more water passes over, which can easily be observed by an absence of bumping. Collect the distillate in a 100 c.c. graduated cylinder, the first 10 c.c. of which are graduated in tenths of a c.c. Rinse out the condenser with naphtha and then read the volume of water which settles to the bottom of the cylinder. This reads directly as a percentage.

Method of Drying Oil.—(1) The crude oil is dried either by warming with calcium chloride in a flask with an inverted glass condenser, and then filtering through cheese-cloth.

(2) By distilling the oil in a copper still until all water has passed over. The distillate which consists of water and light oils is collected in a separating funnel, the lower layer of water drawn off and the light oily layer is added to the residue in the still as the dried sample.

Both of these methods are at fault, however, in that there is a slight loss of the lighter oils, a difficulty that is very hard to overcome.

Analysis of Heavy Naphtha and Distillates.—Sp. gr. at 60° F. is best determined by the Westphal balance. *Flash* and *Fire Tests*. Determine with Tagliabue Cup.

Loss on Spontaneous Evaporation.—Weigh 10 c.c. of the oil from a pipette into a 3½ in. crystallising dish and allow to stand 24 hours at a temperature of 77° F. (25° C.) Weigh again and calculate loss as percentage.

Sulphuric Acid Absorption.—Into a 20 or 30 c.c. carbon tube measure exactly 10 c.c. of the oil and 10 c.c. of conc. sulphuric acid. Mix thoroughly and allow to stand about 30 minutes. Note the amount of oil absorbed by the acid and report as percentage.

Colour is obtained by matching the oil in a 1-in. (or fraction thereof) cell with standard tints, in a Lovibond Tintometer.

Estimation of Hard Asphalt in Mineral Oils by Normal Benzin.—According to L. Allen's Laboratory, Hamburg.[1] A cylinder oil containing 0.2% hard asphalt was treated according to Holde's directions with normal benzin, but the residue did not look like asphalt and was soluble in absolute alcohol, leaving only 0.018% asphalt. A similar removal of paraffin compounds by absolute alcohol from "hard asphalt" residues was observed by Ubbelohde.

[1] *Chem. Rev. Fett-Hars-Ind.*, 20, 192.

Oxygen in Bitumens.—A process has been described by S. P. Sadtler[1] which is a development of that of W. M. Cross, and is carried out as follows: A fused silica combustion-tube 30 in. long is partly filled with iron wool and placed in a combustion furnace.   In one end of the tube is a boat containing the weighed sample of asphalt.   The other end is connected in succession with a U-tube filled with spun glass, and a weighed calcium chloride tube.   The part of the tube filled with the iron wool is strongly heated while that in the vicinity of the boat is kept cool by trickling water over it.   Hydrogen is passed slowly through the apparatus until the calcium chloride tube has attained a constant weight.   The boat is then gradually heated until ultimately it and its contents attain the maximum temperature possible, which is so maintained for a time.   The water formed will be completely carried into the calcium chloride tube if the hydrogen is passed through long enough.   Any sulphur present will unite with the heated iron wool.   The hydrogen used is dried and purified by passing it slowly through concentrated sulphuric acid, over calcium chloride and then over phosphorus pentoxide.

Carbon Test and Ash Residue in Petroleum Lubricating Oils.—Conradson's apparatus[2] consists of an iron crucible or retort having a diameter at top of 80 mm., bottom 45 mm., height 55 mm.   Inside the crucible is placed, upon a support, either a glass crystallising dish (65 mm. in diam.) or a 70–80 c.c. platinum dish; the latter is preferred for further study of the carbon residue. In the lid of the retort are attached two tubes, (1) an exit or delivery pipe 7–8 mm. in diameter, bent and attached to a condenser, and (2) a tube 2 mm. in diameter, for blowing out the heavy fumes.   35 grm. of the oil are placed in the weighed dish, the cover clamped down, using an asbestos washer, and the apparatus put on an asbestos block resting on a tripod and covered with a sheet iron or asbestos hood on top of which is a clay chimney, to distribute the heat uniformly.   To get the upper part of the apparatus hot at the beginning, a large gas flame is used at the start for a few minutes, when it is subsequently lowered; the distillation should be uniform and at the rate of 1 c.c. per minute.   At the end of the distillation, the flame is raised gradually to make the bottom and lower part of the apparatus red hot, when heavy fire test oils are tested, and through the inlet pipe attached to a rubber tubing carbon dioxide or another suitable gas is momentarily blown to expel the heavy, dense fumes or vapours.   After cooling, the dish containing the carbon residue is weighed, the latter ground fine and leached out with boiling water (the solution is tested with litmus paper and with silver nitrate for chlorine); next digested with hot hydrochloric acid (1 : 1), filtered, washed, dried, and weighed.   The difference in weight gives approximately the amount of foreign matter in the carbon residue.   The carbon residue transferred back to the platinum dish is burned off, adding a little ammonium nitrate; if any residue is left it is weighed and deducted from the last carbon weight and

[1] *Orig. Com., 8th Intern. Congress Appl. Chem. (Appendix),* 25, 729–733.
[2] *Orig. Com., 8th Intern. Congr. Appl. Chem.,* I, 131–132.

dissolved in hydrochloric acid. The water and hydrochloric acid solutions are united and examined for impurities (*e.g.*, Fe, Al, CaO, MgO, Na_2O, SO_3). Some lubricating oils contain oleates of lead, aluminium, zinc, calcium or magnesium held either in solution, or suspension or both; before making the above test it should be ascertained if any of these compounds are present.

Proposed Provisional Tests for Lubricants.[1]

Viscosity[2] shall be determined with the Saybolt universal viscosimeter, the dimensions of which are as follows:

Diameter of overflow filling gauge cup	51.0 mm.
Depth of overflow filling gauge cup	13.0 mm.
Diameter of main cylindrical tube	30.0 mm.
Depth from starting head to outlet jet	113.0 mm.
Length of outlet jet	13.0 mm.
Diameter of outlet jet	1.8 mm.
Charging quantity	70.0 c.c.

Tables for the conversion of the readings of other instruments into those of the Saybolt universal viscosimeter are now in preparation by the U. S. Bureau of Standards.

Sp. Gr.—For all practical purposes there is little to choose between the hydrometer, Westphal balance or Geissler pyknometer, provided these instruments are verified. The observations should be taken with the oil at 15.56° (60° F.) and compared with water at the same temperature.

For Baumé hydrometers the committee recommends the use of those based on the formula of the Bureau of Standards:

$$\text{Sp. gr.} = \frac{140}{130 + B\acute{e}. \ 60°/60° \ F.}$$

It should be understood, however, that many Baumé hydrometers now in use are not based on this formula and in converting the readings of any Baumé hydrometer to sp. gr. care should be taken to use the formula on which the instrument in question is based.

Flash and Fire Test.—With the *Cleveland Open Cup.*—The cup is filled. to about ¼ in. from the top and the thermometer is suspended so that the bulb is just immersed in the oil. The oil is heated at the rate of 10° F. per minute by a Bunsen burner with a protecting chimney; as the flash point is approached a test is made for every rise of 3° F. by slowly passing the small bead-like test flame across the cup near the thermometer. The oil should flash near the thermometer when the proper point is reached. The fire test is, as a rule, 50 to 80° F. higher than the flash point. As the open-cup tests are easily affected by draughts they are subject to errors of 5° F. If the thermometer is graduated to read for total immersion, the stem correction should be applied. When this is done it is suggested that "corr." be added to the reading, thus, "flash 379° F. corr."

[1] *Proceedings of the Amer. Soc. for Testing Materials,* 1914. 14, Part I, 358.
[2] The minority report recommended that the Engler method be an alternative method.

Pensky-Martens Test.—Where greater accuracy is required the Pensky-Martens tester should be employed. The method of operating is as follows:

The oil container is placed in a metal heating vessel provided with a mantle in order to protect the heating vessel from loss of heat by radiation. The oil cup is closed by a tightly fitting lid. Through the centre of the lid passes a shaft carrying the stirring arrangement, which is worked by means of a handle. In another opening of the cover a thermometer is fixed. The lid is perforated with several orifices, which are left open or covered, as the case may be, by a sliding cover. This can be rotated by turning the vertical spindle by means of the milled head. By turning this head an opening of the slide can be made to coincide with an orifice in the cover, and simultaneously a very small flame, burning at the movable jet is tilted on to the surface of the oil.

The test is performed by filling the oil into the oil cup up to a certain mark, fixing the cover and heating the oil somewhat rapidly at first, until its temperature is about $30°$ below the expected flash point. The temperature is then allowed to rise only very slowly, by making suitable use of the wire gauze so that the rise of temperature within a half minute does not exceed about $2°$. Every $1.05°$ the milled head is turned and the flame tilted into the oil cup. The temperature at which a slight explosion is produced is noted as the flash point of the oil.

Soap Test.—The test depends upon the fact that the metaphosphates of the earthy and alkali metals and aluminium are insoluble in absolute alcohol. 5 to 10 c.c. of the oil are dissolved in 5 c.c. of $86°$ benzin, and 15 drops of a saturated solution of "stick phosphoric acid" in absolute alcohol are added, shaken and allowed to stand; the formation of a flocculent precipitate indicates the presence of soap. For the accurate determination of these soaps a known quantity of the oil must be ignited and the residue quantitatively examined.

Saponification Value.—This is expressed by the number of milligrams of potassium hydroxide necessary to saponify 1 grm. of the oil. From 2.5 to 10 grm. of the oil, according as 65 to 20% of saponifiable matter are supposed to be present, are boiled with 25 c.c. $N/2$ alcoholic potassium hydroxide in a 200 c.c. Jena Erlenmeyer flask. A reflux condenser is used and the boiling may require from 5 to 8 hours. The excess of alkali is titrated with $N/2$ hydrochloric acid, using phenolphthaleïn. The strength of the $N/2$ potassium hydroxide is determined by boiling 25 c.c. in a similar flask alongside of those in which the oil is treated and for the same length of time.

Alcohol purified with silver oxide according to Dunlap's method should be used, as well as potassium hydroxide "purified by alcohol." For heavy oils, dissolve them in sufficient chemically pure benzol to make a clear solution before adding the potassium hydroxide. Usually 50 c.c. will suffice.

Free Acid.—About 10 grm. of oil are weighed into a 200 c.c. Jena Erlenmeyer flask; 60 cm. of neutral alcohol added, the mixture warmed to about 60° C. and titrated with $N/6$ potassium hydroxide, using phenolphthaleïn, the flask being frequently and thoroughly shaken.

The free acid, if present, should be reported as the number of milligrams of potassium hydroxide necessary to neutralise the acidity in 1 grm. of oil, and the nature of the acid stated if possible.

Sulphur Test.—Proceed as follows: A portion of a sample, 0.7 to 1.0 grm. is burned in a calorimetric bomb containing 10 c.c. of water and oxygen under a pressure of 30 atmospheres. A lower pressure sometimes gives inaccurate results. If the sample contains more than 3% of sulphur the bomb is allowed to stand in its water-bath for 15 minutes after ignition of the charge. In case the sulphur content is as high as 5%, oxygen under pressure of 40 atmospheres is used. With these high pressures in a Berthelot bomb of 500 to 600 c.c. capacity, repeated trials have failed to show even traces of carbon monoxide or sulphur dioxide. If a smaller bomb of about 175 c.c. capacity, such as the Peters or Kroeker, is used, incomplete combustion from a lack of oxygen may result if too large a sample is taken.

After cooling—15 minutes is usually enough—the bomb is opened and its contents are washed into a beaker. If the bomb has a lead washer, 5 c.c. of a saturated solution of sodium carbonate are added, the contents are heated to the boiling point, boiled for 10 minutes and then filtered. This operation is necessary to decompose any lead sulphate from the washer. The united washings are then filtered, acidified with hydrochloric acid, boiled to expel all carbonic acid and the sulphuric acid-content is determined in the usual way with barium chloride.

Gravimetric determination is preferred to volumetric, because the nitrogen contained in the air originally in the bomb is oxidised in part to nitro-acids, which cause a small error if the volumetric determination alone is used. The sulphur content of any combustible material, from light gasolines weighed in a tared gelatin capsule to solid bitumens and cokes, can be readily determined by this method.

This method of burning in a bomb is accurate, practicable and rapid, and is recommended in preference to all of the other methods described. The calorimetric determination, if desired, can be made at the same time (see page 247 for sulphur in asphalt and bitumens).

Test for Water.—Dilute the oil with an equal volume of benzene which has been previously saturated with water. Warm to 32°. Whirl vigorously in a centrifuge until the separated layer of water does not appear to increase in volume.

Alternative Method.—The water content may be determined as follows: 80 grm. of the sample are weighed into a 250 c.c. distilling flask and an equal quantity of xylol or benzin, having an initial boiling point of about

150°, is added. . The distillation is carried out at the ordinary rate of 1 drop of distillate per second until 163° is reached. In the event of a few drops of water adhering to the condenser and failing to run into the receiver, t hey may be removed with a weighed pellet of cotton and the amount of water so obtained added to that found in the distillate.

Precipitation Test.—Dissolve 10 c.c. of oil in 90 c.c. of 86 to 88° benzin (from Pennsylvania crude) in a tapered graduated tube. Whirl in centrifuge until no further change in the amount of precipitate is observed.

Microscopical Examination.—Put a few drops of the well-mixed oil on a slide and note the nature of the suspended matter—whether carbonaceous specks, flakes of paraffin which disappear on warming, or foreign matter. Polarised light is a great aid in detecting paraffin crystals, showing them white
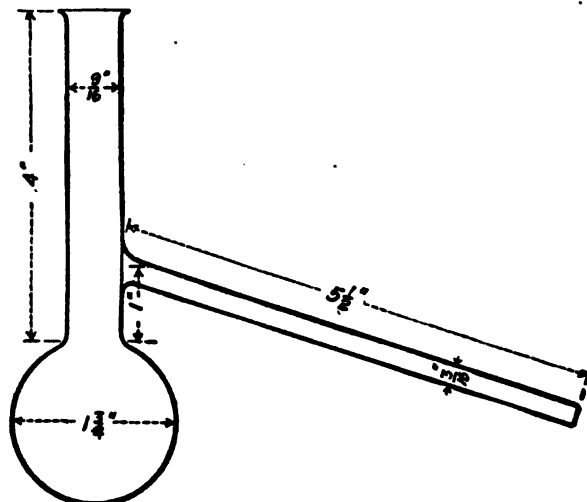
Fig. 12.—"Gray" Carbon Residue Flask.

on a black background. The polariscope is excellent for this same purpose, showing them when it is impossible to see them with direct light.

Carbon Residue Test (T. T. Gray's Method).—To a tared 1-ounce flask of the dimensions shown in Fig. 12 preferably of quartz, add 25 c.c. of the oil to be tested and weigh. Wrap the neck of the flask with asbestos paper as far down as the side arm. Stopper tightly with a good cork. Connect to a small aerial condenser by plugging the space with asbestos or glass wool. Provide a shield which will protect the flame and the flask up to the side tube. Using the flame of a good Bunsen burner, heat the flask so that the first drop of distillate will come over in approximately 5 minutes. Continue the distillation at such a rate that 1 drop per second will fall from the end of the condenser. As the end of the distillation approaches, increase the heat just enough so that the heavy vapors are allowed to con-

dense and drop back into the flask; continue increasing the heat until the flask is enveloped in the flame, and maintain the temperature 5 minutes. Allow the flask to cool, remove the asbestos covering and cork, and burn out completely the carbon and oil in the neck as far down as the side tube and in the side tube. Heat the bottom of the flask until no more vapours are given off, cool and weigh.

The viscosity of lubricating oils is measured by the Ostwald viscosimeter described in text-books of Physcial Chemistry (*e.g.*, Findlay's Practical Physical Chemistry).

Filtered oil is run into the viscosimeter to the marks *c-d*. It is now suspended in a bath of paraffin wax or suitable clear high flash oil. It is adjusted quite vertically by reference to plumb lines suspended in the bath. A 2-litre Jena beaker forms a convenient bath, and stirring may be carried out by means of a glass paddle, run by a hot-air motor. A delicate thermometer is immersed in the bath. If the determinations are carried out in a suitable place, well shielded from draughts of air, steady temperatures may be maintained for long intervals. It is advisable, however, to guard the apparatus with asbestos millboard as a protection against temperature fluctuations. After the viscosimeter has been immersed 15 minutes at constant temperature, the levels of the oil should be accurately adjusted to the marks *c-d*, by means of a warmed glass pipette. Rubber tubing is fixed to the top of bulb *A*, and the oil slowly sucked up above mark *a*. It is then allowed to flow down and when the meniscus passes *a*, a stop-watch reading to fifth seconds is started and the time of flow between *a* and *b* is taken. Four or five determinations are made and averaged. For ordinary oils the following dimensions will be found useful: Capillary, *C*, 6 cm.×1 mm. bore; bulb *A*, 4 c.c.; bulb *B*, 8 c.c.; length over all 15 cm. The viscosimeters may be obtained from Messrs. Müller, Orme, & Co., High Holborn, England.

Specifications for Gas Oils for Railways.—The most important characteristic of the oil is its "heat yield" (the product of the yield of gas and the heating value of the gas). This is nearly independent of the temperature of gasification. It varies with the hydrogen content of the oil; but the loss on compression of the gas depends on the hydrogen content of the oil. Since for railway purposes the gas is always compressed, it is the "heat yield" of the gas after compression which is of interest. Where the gas is to be used in open flame burners, the "light yield" (candle power yield) is of more importance, but this is commonly not the case in railway lighting. Of impurities in the oil, sulphur, water, and creosote are to be controlled; nitrogen and oxygen are of no importance. In sampling deliveries, representative samples for water are exceedingly difficult to obtain. By gently heating a sample of the oil it is freed from water, the sp. gr. of this taken, the volume of oil de-

[1] Landsberg, *J. Gasbel.*, 56, 10-14.

livered (as shown by measurement or a meter), multiplied by this sp. gr. and the resulting weight subtracted from the shipper's weight is taken to give the water. For determining the "heat yield," a 1-hour actual run, requiring 300-450 kg. oil, is sufficient.

Transformer Oils.[1]—A good transformer oil must have a large insulation capacity. It must be absolutely free from water, be neutral, and to be safe must have high flash and solidifying points. The bath in which the insulation capacity is determined is made of glass, insulated from the earth by porcelain strips resting upon a glass plate; the electrodes are of brass, fitted with porcelain clasps at the point through which they pass the hard gum cover of the bath. Three freshly refined oils with viscosities ranging from 3.8° to 20° (Engler) at 20° were examined. With the electrodes 5 mm. apart, and voltage of 10,000, no spark passed after 20 minutes. When the voltage was increased gradually to 40,000 a spark passed through the heaviest oil after 5 seconds, through a lighter oil after 30 seconds, and through the lightest oil after 60 seconds. The insulation capacity increases with viscosity. The oil loses its insulation power with usage. An oil with a viscosity of 8° (Engler) at 20°, which had been in use for 3 years, allowed no passage of a spark at 10,000 volts, but did so immediately at 17,000. When first tested this oil allowed no passage at 18,000 volts after 5 minutes. The changes in temperature cause the oil to darken and a black sediment is deposited. This powder (sp. gr. about 1) is soluble in benzene, ether, carbon disulphide and concentrated sulphuric acid, but is insoluble in benzin, alcohol, and lubricating oil; it does not melt, it cokes on heating, and corresponds to a highly oxidised asphalt. The formation of this resinous matter is undesirable on account of the possibility of a short circuit. The asphaltic content of a transformer oil is determined by passing oxygen through the oil heated to 120° for 70 hours, dissolving the oil in benzin, and filtering the insoluble matter on a weighed filter. The formation of this resinous matter depends chiefly upon the degree of refining, and upon the viscosity of the oil. An oil with a viscosity of 6° Engler at 50°, unrefined, gave after heating for 70 hours, and passing oxygen through the oil, 1.09% of resinous matter; when refined with 10% of sulphuric acid only 0.036% of insoluble matter was obtained, and, when refined with 10% sulphuric acid and decolourised with 4% of sodium silicate, but 0.019% was obtained. The conclusion is reached that a light spindle oil with a viscosity of 3° to 5° at 20°, and 150°-160° flash point is the most desirable for a transformer oil. The disadvantage of the greater evaporation must be weighed against the advantages of a large insulation capacity, but slight decomposition, and cheapness.

Evaporation Test for Mineral Lubricating and Transformer Oils (U. S. Bureau of Standards).—In order to obtain comparative results in the evaporation test of mineral oils, the same weight of oil must always be heated in vessels

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[1] P. Breth, *Petroleum*, 7, 290-291.

of the same size, so that the oil surface shall always be the same in area and the convection effects be alike. Brass vessels are preferable to those of glass, because they can be made with their walls and bottoms of the same thickness, thus insuring more uniform heating. A convenient size is 5 cm. in internal diameter with sides 3 cm. high. Tubing of this size with a wall thickness of 0.75 mm. can be bought. The bottoms may be made of sheet brass not more than 0.5 mm. thick. It is best to use silver solder, so that the heating need not be limited to the lower temperatures. A vessel of the size indicated weighs somewhat less than 42 grm.

A convenient weight of oil is 5.0 grm. To avoid smearing oil on the walls of the tubes the writer used a small pipette with a 2 cm. stem below the bulb. The final adjustment of the weight was made by just touching an oily or a dry stirring rod to the surface of the oil. It is comparatively easy to weigh out the oil within 0.5 mg. of the amount desired, though a much larger variation would be of little consequence in calculating the percentage of evaporation.

In a neutral atmosphere there might have been somewhat greater losses, and possibly still greater differences between the losses with increasing diameter of tube, than the amounts found. In the air there is more or less oxidation, partly involving loss of carbon dioxide and water, but mainly due to the formation of compounds containing carbon, hydrogen and oxygen, which are precipitated in part from the oil as a fine, brown sediment. The observed losses are really the sum of volatile oil, carbon dioxide and water lost, minus the oxygen taken up.

It would be interesting to repeat the work herein described using an atmosphere of carbon dioxide, nitrogen or steam instead of air, but the results would be of less general application.

Estimation of Paraffin in Mineral Oils (According to Schwarz and von Huber).[1]—1–5 grm. of distillate (less for paraffin pulp) are dissolved in 20 c.c. of 0.812 butanone in a test-tube of 25 mm. diameter at room temperature. After cooling to −20° more butanone is added to keep all oil just dissolved. The precipitate is dissolved in benzin, evaporated and weighed. The washing is effected with four to five portions of 5–10 c.c. of 0.812 butanone. Comparison with the Engler-Holde method shows good agreement, the latter showing sometimes low results by 1% when high in paraffin on account of its solubility in the alcohol-ether mixture used in the Engler-Holde method.

Analysis of Mixtures of Ceresin and Paraffin.—Since ceresin, made by chemical treatment from Galician ozokerite, costs about six times as much as distilled paraffin, methods of separation have been worked out by N. Chercheffsky[2] to be applied to the unsaponified residue obtained in the ordinary way in fat analysis. It was assumed that the melting point of commercial ceresin is fairly constant about 69°, and that the most likely adulterant is

[1] *Chem. Rev. Fett-Hars-Ind.*, 20, 242–244.
[2] *Mat. grasses*, 4, 2235–2239; *Seifensieder Ztg.*, 38, 986–987.

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paraffin of melting point about 52°. (1) Solubility in carbon disulphide (paraffin, 19.07 grm.; ceresin, 1.97 grm. per 100 c.c. at 15°) and in carbon tetrachloride (paraffin, 11.78; ceresin, 1.95). (2) Critical solution temperature: this is said to be almost independent of the proportion of solvent and substance used; it is the temperature, at which a mixture, heated in a sealed tube until the meniscus of separation becomes horizontal, becomes turbid on being shaken and allowed to cool. Alcohol of 96.5% is recommended as solvent; in this case ceresin becomes turbid at 172.5° and paraffin at 148°. Various known mixtures of ceresin and paraffin gave results within 0.6° of calculated temperatures. (3) Temperature of turbidity, method of determination same as last, except that the tube is open. In case of benzene which is recommended as solvent for this test, the temperature is 47.4° for pure ceresin, and 24.7° for paraffin. (4) The index of refraction of pure ceresin at 78° is 1.4352, at 100°, 1.4268; of paraffin, 1.4280 and 1.4185 respectively. In all cases, the values obtained with mixtures are approximately arithmetically proportional to the values of the pure constituents.

G. Armani and G. A. Rodano[1] recommend for the detection and approximate determination of ceresin in admixture with paraffin a method based upon the difference in the critical temperatures of solution of the two substances in a mixture of equal volumes of absolute alcohol and benzene. 0.1 grm. of the sample is dissolved in 10 c.c. of the solvent and the hot solution is allowed to cool slowly, the temperature at which a turbidity or separation of the dissolved substance is observed being noted. For pure ceresin the critical temperature is 50°, whilst for mixtures of ceresin and paraffin containing the per cent. of ceresin given, the critical temperatures are: 90, 48°; 80, 47.5°; 70, 47°; 60, 44.5°; 50, 43°; 40, 41.5°; 30, 40°; 25, 38°; 20, 36.5°; 10, 30°; and 5% of ceresin, 27°. With paraffin alone, the critical temperature of solution is usually about 25°, but may vary in different kinds from 20° to 28°. In all cases examined, however, of mixtures of paraffin with 10% ceresin, the critical temperature was 30°. In applying the method to candles, 0.1 grm. of the sample is tested as described above; if the critical temperature is below 28°, the presence of an appreciable quantity of ceresin is excluded. If a higher critical temperature is observed, a portion of the sample is subjected to repeated saponification with an aqueous alcoholic solution of potassium hydroxide (40 grm. dissolved in a mixture of 100 c.c. of water and 200 c.c. of alcohol), and 0.1 grm. of the unsaponifiable matter is tested as described above in order to determine approximately the proportion of ceresin present.

Paraffin.—Weigh out 50 grm. of the oil into a tared iron retort. The cover of the retort is bolted on to the latter with a gasket of heavy wrapping paper separating the two. The distance from the retort to the receiver must

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[1] *Ann. Lab. Chim. Centr. delle Gabelle*, 1912, **6**, 109–118.

be at least 30 in., 20 in. of iron tubing and a 10-in. glass tube. Distill so that all distillate passes over in not more than 25 minutes.

Collect the distillate in a 3-ounce tin box. The glass tube is heated in order to remove any adhering distillate into the receiver. Weigh the retort after it has cooled to obtain the weight of the coke. The distillate is warmed in order to dissolve the thick oils and get an average sample. Now weigh out 5 grm. of the distillate into a small Erlenmeyer flask, add 25 c.c. ether and 25 c.c. absolute alcohol and freeze in an ice and salt mixture together with a wash bottle containing alcohol ether (1:1) mixture for 40 minutes. At the same time a funnel is prepared which is also surrounded with the freezing mixture. At the expiration of the required time filter the paraffin on a hardened filter paper using suction, washing out the flask and the paraffin with alcohol ether until free from colour. Remove the paper from the funnel, scrape off the paraffin with a knife into a tared crystallising dish and dry at 100° until all alcohol ether is dissipated. Cool and weigh. Calculate per cent. paraffin in the distillate, then in the original oil by subtracting per cent. coke from 100% and multiplying the per cent. paraffin in the distillate by this figure.

Vaseline Oil for Internal Use.—According to A. Vicario[1] vaseline oil or liquid paraffin is obtained from petroleum oils poor in vaseline,. and is expressed in the process of preparing solid paraffin. As required by the French Codex it should be of Caucasian origin and consist of hydrocarbons of the series C_nH_{2n}. By comparison of the rate of flow through a fine tube at the ordinary temperature it should have a viscosity 8–19 times greater than that of water. The sp. gr. varies between 0.840 and 0.890. Acids and fatty substances should be absent. American oils which are in some cases less pure and limpid than the Russian oils have a sp. gr. of 0.870–0.945, and give a yellow to a black colouration when shaken with concentrated sulphuric acid till the latter ceases to be coloured. The oily layer is then separated, washed with sodium carbonate solution, dehydrated with anhydrous sodium carbonate and filtered. When subjected to artificial digestion experiments under varying conditions, the oil was unattacked. American oils have been recently improved and these oils should act like Russian oils in the sulphuric acid test.

Cyclic Hydrocarbons.

Testing Commercial Benzols.—Owing to the increasing demand for pure toluene benzol is produced of great purity at a minimum cost. Therefore its analysis is relatively simple. The distillation test in Vol. III, p. 208 is quite satisfactory for the testing of such pure products (see *Methods of Technical Analysis* by Lunge-Keane, 2, p. 784). See also pages 230 and 234.

A reliable method for the assay of commerical benzols, which is in general

[1] *J. Pharm. Chim.*, 1914, **9**, 149–154.

use in Germany, is the result of a proposal due to Lunge[1] for the attainment of uniform methods of analysis for benzols. A spherical vessel 66 mm. diam., made of strong copper 0.6 to 0.7 mm. thick, which is slightly flattened at the bottom, is used for the distillation. It has a neck 25 mm. long, the diameter of which is 22 mm. at the top and 20 mm. at the bottom, for the reception of the glass prolongation, which is 150 mm. long and 14 mm. wide. In the middle of the glass portion is a bulb of 30 mm. diameter, above which, at a distance of 10 mm., a side-tube of 8 mm. diameter is sealed almost at right angles. The glass column and the spherical vessel are connected by means of a good cork stopper. A Bunsen burner of about 7 mm. diameter or a benzin spirit lamp is used for heating. The burner is placed in a tinplate cylinder provided with a door, and also with four round holes 10 mm. above the bottom, and four more 10 mm. below the top, for ventilation. This small heater is covered at the top with a sheet of asbestos having a round hole of 50 mm. diameter in which the spherical retort is placed. The inner tube of the glass Liebig condenser has a length of 800 mm., and is inclined with the outlet 100 mm. below the level of the inlet. The thermometer is made of thin glass, and is graduated in $\frac{1}{10}$ths of a degree for pure benzene, and in $\frac{1}{5}$ths of a degree for commercial products; it should be about one-half the diameter of the neck, and must be so fixed that its bulb is exactly in the middle of the widened part, and should be compared from time to time with a standard thermometer. The distillate leaves the condenser through a bent adapter, and runs down the sides of a 100 c.c. graduated cylinder.

To carry out the valuation, 100 c.c. of the liquid are put into the flask and the distillation is conducted so that 5 c.c. distil over per minute, *i.e.*, 2 drops per second; it is complete when 95 c.c. have distilled over. In order to correct any cause of error due to different barometer readings, the method of Bannow can be employed, in which the thermometer reading is checked by distilling 100 c.c. of water in the same vessel and observing the thermometer reading at the boiling point of the water, when 60 c.c. of the water have distilled over. It is simpler, however, to utilize Lender's corrections as follows:

(1) When the barometer stands at 720 to 780 mm. the percentages of the distillate obtained are reduced to the corresponding amounts for the normal reading of 760 mm. by applying the following corrections:

For 90 % benzol, 0.033 %.
For 50 % benzol, 0.077 %.

to be added or subtracted for each millimetre of difference.

(2) For barometer readings of from 720 to 780 mm. for distillation up to 100°, for each millimetre difference add or subtract:

For 90 % benzol, 0.0453°.
For 50 % benzol, 0.0461°.

[1] *Chem. Ind.*, 1884, 1, 50.

(3) In the case of pure products, the difference of temperature for each millimetre difference in the barometer readings between 720 mm. and 780 mm. is:

Benzene, about 0.043°.
Toluene, about 0.047°.
Xylene, about 0.052°.

Example.—In the case of a 90% benzol, 88.8% distilled over at 100°, the barometer reading being 721.2 mm. to reduce the percentage to the standard reading of 760 mm.:

760.0 − 721.2 = 38.8 mm.
38.8 × 0.033 = 1.28 %.
88.8 − 1.28 = 87.52%.

at 100°.

Or, if the distillation of the same benzol be carried out at 730 mm. barometer reading, then according to (2):

760 − 730.0 = 30.0 mm.
30 × 0.0453 = 1.359°.

In carrying out this distillation, therefore, in order to obtain the percentage distilled over, when the height of the barometer is 760 mm., the temperature of distillation must be taken at 100−1.359=98.641°, or approximately 98.6°, instead of 100°.

A. Spilker has shown, however, that the following artificially prepared mixtures all give the limits of boiling point required for a 90% benzol:

(a) 82.0% benzene + 18.0% toluene.
(b) 92.2% benzene + 7.8% xylene.
(c) 90.0% benzene + 5.0% toluene + 5.0% xylene.
(d) 84.0% benzene + 13.0% toluene + 3.0% xylene.

Of these mixtures only (d) corresponds in composition with an average commercial 90% benzol.

Method of Testing Drip Oils[1].

Apparatus:

The apparatus shall consist of the following standard parts:

I. Flask:

The distillation flask shall be a standard 100 c.c. Engler distilling bulb, having the following dimensions (see Stillman's Engineering Chemistry):

Angle of tubulure.................................... 75.0°
Diameter of bulb.................................... 6.5 cm.
Length of neck..................................... 15.0 cm.
Diameter of neck.................................... 1.6 cm.
Surface of oil to tubulure........................... 9.0 cm.
Length of tubulure.................................. 10.0 cm.

A variation of four per cent. (4%) from the above measurements is allowable.

II. Thermometer:

Gas-filled centigrade thermometer constructed according to the following specifications:

[1] Owing to the high cost (at this time of writing) of benzol and toluol, the specifications for testing drip oils are very rigid. The method given is much used by the Reviser of this Section and was supplied him by C. C. Tutweiler.

1. To be made of Corning or Jena glass.

2. Diameter of stem not less than 6.5 mm. nor more than 8.5 mm.

3. Length of the thermometer not less than 335 mm. nor more than 400 mm.

4. Length of thermometer between 0° mark and 220° mark not less than 230 mm. nor more than 245 mm.

5. Length of bulb to capillary not less than 12 mm. nor more than 16 mm.

6. Diameter of bulb at centre of same not less than 5.25 mm. nor more than 6.25 mm.

7. Mercury column to rise from 10° to 95° in not more than 6 nor less than 4 seconds when plunged into boiling water.

8. To be correct within $\frac{1}{10}°$ at 100° and at 200° when tested against a similar thermometer certified by the Bureau of Standards.

9. Arranged for $3\frac{1}{2}$ immersion with graduations starting above immersion line.

III. Condenser:

Liebig glass condenser and tube as follows:

Length of body of jacket	300–400 mm.
Width of body of jacket	25– 40 mm.
Length of inner tube	450–500 mm.
Width of inner tube, which is to be either straight or provided with an enlarged upper end, between 12 and 25 mm. at each end.	
Width of end of inner tube	12– 35 mm.

IV. Stands:

Two iron stands provided respectively with one universal clamp for holding the condenser, and one light grip arm with cork-lined clamp for holding the bulb.

V. Burner:

A Bunsen burner shall be employed.

VI. Cylinders:

Glass cylinders of 25 c.c. capacity graduated to $\frac{1}{5}$ c.c. shall be used in collecting and measuring the fractions distilling up to 135°C.

Setting Up Apparatus.

The apparatus is set up so that the top of the bulb of the thermometer is opposite the middle of the tubulure. All connections should be tight.

Distillation Test.

One hundred cubic centimetres of the oil measured at 15.5°C. are placed in the bulb and, after adjusting the thermometer, condenser, etc., the distillation is commenced, the rate being so regulated that the stream flows at the maximum rate possible while producing distinct drops from the end

of the condenser tube. Cold water should be passed through the condenser during the distillation. The receiver is changed as the mercury column just passes the 135°C. fractionating point.

Fractions.

The distillate shall be collected in two fractions. One fraction representing the amount distilling to 135°C.; the other representing the amount distilling above 138°C. The amounts coming over, up to the following points should be noted, reading to 0.1 c.c. at 135°C. and 1 c.c. at the other points.

100°C.	150°C.
110°"	160°"
120°"	170°"
130°"	180°"
135°"	190°"
140°"	200°"

All fractions, before being read, must be brought to 15.5°C.

Water.

Should the distillate coming off to 135°C. contain water, the water and oil shall be allowed to separate by subsidence and the amount of water and oil shall be read separately to one-tenth cubic centimetre (0.1 c.c). The observed amount of the oil fraction shall then be corrected by dividing the same by the figure obtained by deducting the per cent. of water as regards volume of the 100 c.c. sample from 100%. The result shall be taken as being the corrected amount of oil distilling to 135°C.

Estimation of Aromatic Hydrocarbons in Admixture with Paraffins.—According to Kraemer and Spilker,[1] this estimation is carried out as follows: 200 grm. of the sample are treated in a capacious separating funnel with 500 grm. of fuming sulphuric acid containing 20% of anhydride, and shaken for a quarter of an hour, avoiding undue heating; the whole is then allowed to stand for 2 hours. The layer of sulphuric acid is drawn off and the operation repeated twice with the same quantities of fuming acid. After treatment with 1,500 grm. of acid as above, everything except the paraffins, carbon bisulphide and naphthenes has usually dissolved. The oil remaining in the funnel is collected and the whole of the acid used is poured, with shaking, upon an equal weight of chopped ice contained in a 3-litre flask, taking care that the temperature does not rise above 40°. The solution is then boiled over a naked flame and the free hydrocarbons present are distilled off and collected in a 100 c.c. separating funnel, the distillation being continued until 50 c.c. of water have passed over after the oil. In this manner all the oil dissolved by the sulphonic acids or mechanically mixed with the liquid is recovered and, after the water has been drawn off, is added

[1] Muspratt, *Handbuch der technischen Chemie*, 4th ed., Vol. 8, p. 43.

to the main quantity. The whole of the oil is then again repeatedly shaken with 30 grm. of fuming sulphuric acid containing 20% of SO_3, until no further reduction in volume takes place, after which it is finally washed with a small quantity of water. The weight of oil obtained, divided by 2, gives the percentage of paraffins in the sample. This is scarcely more than 1% in the case of 90%, 50% and 0% benzols; toluol usually contains none; xylol, on the contrary, often contains up to 3% of paraffins.

Estimation of Unsaturated Hydrocarbons in Aromatic Hydrocarbons.—A measure of the quantity of unsaturated compounds (hydrocarbons of the ethylene and acetylene series, etc.), present in a crude benzol is afforded by titration with bromine. The bromine water formerly used for this purpose is best replaced by a solution of potassium bromide and bromate, which on addition of sulphuric acid, sets free 8.0 grm. of bromine per litre. The test is carried out by Frank in the following manner: 5 c.c. of the sample are added to 10 c.c. of dilute sulphuric acid (1:5), contained in a 50 c.c. vessel provided with a glass stopper, and $N/10$ bromide-bromate solution, containing 9.9167 grm. $KBr + 2.7833$ grm. $KBrO_3$ per litre, is added from a burette until, after 5 minutes, ·shaking, bromine still remains. The titration is finished when the floating oil remains orange-red in colour after standing for 15 minutes and when 1 drop gives a dark blue colouration with freshly prepared potassium iodide-starch paper. The quantity of bromine used (1 c.c. = 0.008 grm. Br) should be given as such. To obtain accurate results, a preliminary estimation is first made, and then two exact estimations, the mean of which is taken. Pure benzene and pure toluene should show a marked bromine reaction after the addition of 0.1 c.c. of the bromine solution, whilst 90 and 50% benzols will decolourise, on an average, 0.6 c.c. of the reagent, but seldom more than 1 c.c. Commercial xylene absorbs 2% of bromine in a few minutes and considerably more on standing for some time.

Page 154, line 1, after "Viscosimeter" insert "(Fig. 7)."

Page 154, lines 23 to 25, delete "The cut was loaned by Bullock and Crenshaw of Philadelphia" and "shown in Fig. 6."

Page 174, line 6, for "or the radius" read "r, the radius."

Page 176, line 15 from bottom, insert "is" after "crucible."

Page 178 line 20 for "K_2PtCl_2 read "K_2PtCl_4."

Page 179, line 4 from bottom, for "generally" read "general."

Page 198, in table for "Naphthaline" read "Naphthalene," for "Renene" read "Retene."

Page 199, in table systematic name for cymene should be "methylisopropylbenzene" not "methylpropylbenzene."

Page 209, line 10, for "in" read "of."

Page 210, line 5 from bottom, for "tetra-p-quinonisol" read "p-methoxytetrahydroquinoline;" and in the same line for "be shaken into" read "and."

Page 224, line 8 from bottom, for "nitrofication" read "nitration."

Page 228, line 2, for "fullows" read "follows." Line 20 from bottom, for "$CS_2(C,H_5NH.NH_2)_2$," read "$CS_2(C_6H_5NH.NH_2)_2$," and delete comma after phenylhydrazine.

NAPHTHALENE AND ITS DERIVATIVES.

By W. A. DAVIS.

Naphthalene in Coal Gas.—Several papers dealing with the estimation of naphthalene in coal gas have been published since the appearance of Vol. III. In all cases the methods proposed for the purpose differ only slightly from those described in this volume.

Albrecht and Müller[1] recommend a modification of Rutten's process[2] which is itself a modified form of Colman and Smith's method. 2.5 grm. of picric acid are placed in approximately equal parts in 2 wash bottles of 100 c.c. capacity, 25 c.c. of water are added to each bottle and the contents shaken until the water is saturated and the 2 wash bottles are connected with the glass tubes in contact so as to prevent the gas from acting on the rubber connection. The gas is passed through the solution at the rate of from 40 to 50 litres per hour until from 0.05 to 0.2 grm. of naphthalene has been apparently absorbed. The contents of the flasks are then washed into a 250 c.c. measuring flask and the free picric acid dissolved by adding water and gently warming. After cooling the solution is diluted to 250 c.c. and 200 c.c. are titrated with $N/10$ potassium hydroxide using lacmoid as indicator. At the same time a similar quantity of a solution containing 2.5 grm. of picric acid in 250 c.c. is titrated similarly; the difference in the number of c.c. of potassium hydroxide required multiplied by the factor $\frac{5}{4} \times 0.0128$ gives the quantity of naphthalene present in grams. The accuracy of the method is shown by means of tests in which known weights of naphthalene were volatilised in dry nitrogen gas. In applying the method to coal gas, care must be taken to remove tar, cyanogen, hydrogen sulphide and ammonia. The use of scrubbing apparatus containing wood shavings, moist iron ore, etc., is shown to give quite unreliable results. Good results, however, are obtained by simply washing the gas in 2 wash bottles containing dilute sulphuric acid followed by two containing dilute potassium hydroxide.

From tests made by the authors cited, the higher percentage of naphthalene in crude gas from vertical retorts as compared with horizontal retorts (10.7 grm. per cubic inch, with a gas temperature of $71°$ compared with 8.6 grm. per cubic inch with a gas temperature of $54°$ for English coal and 8.6 grm. per cubic inch with a gas temperature of $60°$ for Upper Silesian coal) is due simply to the higher temperature and is not dependent on the class of coal employed.

[1] *J. Gasbeleucht.*, 1911, **54**, 592.
[2] *J. Gasbeleucht.*, 1909, **52**, 694.

A. Wein[1] has proposed the following method of working: about 40–50 litres of the gas are drawn by a filter pump through a solution of picric acid contained in a set of 3 washing flasks, which are kept well cooled during the absorption. The gas passes from the last flask through a cotton-wool filter, then through a flask containing concentrated sulphuric acid and thence to the meter and the filter pump. About 700 c.c. of picric acid solution containing 12 grm. per litre are used. The connection between the first and second flask is provided with a by-pass so that after 20–25 litres have been passed the gas can be admitted directly to the second flask. When sufficient gas has been passed, air is sucked through so as to sweep out all naphthalene from the tubes, the flasks are then closed and heated for 30 minutes in a water-bath at 40°. After cooling, the whole of the precipitate consisting of naphthalene picrate contaminated with tar and ammonia is collected on a filter and placed together with the filter paper in an Erlenmeyer flask with 500 c.c. of water acidified with sulphuric acid so as to fix the ammonia. The whole is boiled gently so as to decompose the naphthalene picrate and the naphthalene is drawn with a current of air through flasks containing a picric acid solution of known strength. These flasks are then closed and heated at 40° and after cooling, the naphthalene picrate is filtered off and the picric acid in the filtrate estimated volumetrically. For this purpose, 50 c.c. of the solution are mixed with 10–12 c.c. of a solution containing 100 grm. of potassium iodide and 30 grm. of potassium iodate per litre and the iodine liberated according to the equation

$$\text{KIO}_3 + 5\text{KI} + 6\text{C}_6\text{H}_2(\text{NO}_2)_3\text{OH} = 6\text{C}_6\text{H}_2(\text{NO}_2)_3\text{OK} + 3\text{H}_2\text{O} + 6\text{I}$$
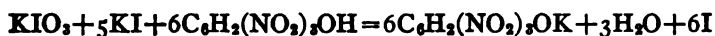
is titrated with sodium thiosulphate. Wein's process seems to be more tedious and to present few, if any, advantages over the ordinary method of working.

Schlumberger[2] has also suggested a modification of Colman and Smith's method which he states is quite satisfactory. Exactly 2.7 grm. of picric acid are placed in a 10-bulb absorption tube and made up with water to about 100 c.c. The gas is passed through saturated citric acid to free it from ammonia, then through the absorption tube and finally through the meter. 300–400 litres of gas should pass in 10–15 hours. The contents of the tube are washed into a 250 c.c. flask, diluted to the mark and, after stoppering, the flask is heated 30 minutes at 40° with frequent shaking and then cooled. The contents are filtered through asbestos and the picric acid in 100 c.c. of the filtrate determined by adding 25 c.c. of potassium iodide-iodate solution (150 grm. iodide, 30 grm. iodate, 400 c.c. of water) and estimating the liberated iodine with thiosulphate. 2.7 grm. of the same picric acid have been previously dissolved in 250 c.c. of water and 100 c.c. titrated in the same way. The difference between the 2 titrations gives the picric acid combined

[1] J. Gasbeleucht., 1911, 54, 891.
[2] J. Gasbeleucht., 1912, 55, 1257.

with the naphthalene. The presence of benzene in the coal gas does not interfere with the estimation of naphthalene. Schlumberger gives data for the vapour pressure of naphthalene at different temperatures between 0° and 50° and the corresponding number of grams of naphthalene per 100 cubic metres.

Temperature	Vapour pressure in millimeters of mercury	Grams of naphthalene per 100 cubic metres
0	0.006	4.51
5	0.010	7.38
10	0.021	15.23
15	0.035	24.95
20	0.054	37.83
25	0.082	56.48
30	0.133	90.10
35	0.210	139.96
40	0.320	209.88
45	0.518	334.39
50	0.815	517.94

Laurain[1] describes two methods, in use at the Paris gas works, of estimating the amount of naphthalene in coal gas. Both depend upon direct cooling and condensation and are not chemical in their nature; being open to objection on different grounds they are not described here.

Estimation of Naphthalene in Spent Oxide.—According to W. C. Davis[2] the separation of naphthalene from spent oxide by the process proposed by White and Ball,[3] which consists in volatilising the naphthalene in a current of hot air, gives very low results, owing to the incomplete volatilisation of the hydrocarbon. It is better to separate the naphthalene by steam distillation and then to estimate it by the picrate process. The details are as follows: 10 grm. of the sample are weighed into a Wurtz flask and subjected to steam distillation, the steam being passed through a second Wurtz flask, heated to 100° and containing 50 c.c. of N-sulphuric or citric acid, then into a condenser and finally into a receiver consisting of a wide-mouthed bottle. The whole apparatus is sealed by placing at the exit a wash bottle containing 25 c.c. of a saturated picric acid solution. The distillation is complete in about 10 minutes. The naphthalene in the condenser is then melted out, the picric acid in the final washing flask is washed into the receiver and sufficient solid picric acid added to make the whole solution saturated; the receiver is closed with a rubber stopper and heated in the water-bath until a clear solution is obtained. The solution is cooled, with occasional shaking, and the excess of picric acid estimated by titrating with N/10 sodium hydroxide in the usual way.

It is necessary, as indicated in the details given above, to have the solution containing the naphthalene saturated with solid picric acid before heating to convert the naphthalene into picrate; unless this precaution is observed, slightly low results are obtained, owing to the solubility of naph-

[1] J. Gas Lighting, 1912, 118, 984.
[2] J. Soc. Chem. Ind., 1914, 33, 1120.
[3] J. Gas Lighting, 1904, 88, 262; Lunge's Technical Methods of Chemical Analysis, ii, 803.

thalene picrate in water. Experiments made with weighed quantities of pure naphthalene showed that the process described above is sufficiently reliable for all practical purposes.

Estimation of β-Naphthol.—J. M. Wilkie[1] has published a careful investigation of Messenger and Vortmann's iodometric method (Vol. III, p. 258) as applied to the estimation of phenols. In the case of β-naphthol, when its cold aqueous solution is treated with $N/10$ iodine an almost quantitative yield of 1-iodo-2-hydroxynaphthalene is obtained; but in the presence of a slight excess of alkali hydroxide over that corresponding with the formation of $C_{10}H_7ONa$, a crystalline colourless product is no longer obtained but the green amorphous compound of Messenger and Vortmann. In carrying out the quantitative process, variable results are obtained with β-naphthol according to the excess of iodine used, a result which differs from that obtained with phenol and salicylic acid, in which cases the absorption of iodine is independent of the alkali and iodine, provided that the excess exceeds 60%. The quantitative process is, however, quite satisfactory when carried out in *practically neutral* solution as follows:

1.44 grm. of the naphthol is dissolved in 10 c.c. of $N/1$ sodium hydroxide; if the solution is diluted to 200 c.c. it is $N/10$ to iodine. 10 c.c. are then transferred to a Jena glass bottle, 150 c.c. of water added at the ordinary temperature and 4 c.c. of $N/10$ sulphuric acid so as to leave a feebly alkaline solution. 20 c.c. of $N/10$ iodine are added and after standing in a bath of water at 55° to 65°, some sulphuric acid is added and the residual iodine titrated with thiosulphate in the usual manner.

Under the conditions given the interaction takes place practically quantitatively according to the equation:

$$C_{10}H_7OH + I_2 = C_{10}H_6I.OH + HI$$
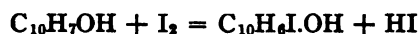
Pharmacopœia Requirements.—The British Pharmacopœia, 1914, gives the melting point of β-naphthol as 122°, and the following test for the absence of α-naphthol; 0.1 grm. dissolved in 10 c.c. of boiling water yields with 10 drops of an aqueous solution (1 in 30) of ferric chloride a white precipitate which becomes brown but not violet. The pure naphthol should give no appreciable ash.

ERRATA IN VOL. III.

Page 250, line 21, "pages 274 and 275" should read "pages 245 and 276."
Page 258, line 19 "page 274" should read "pages 245 and 276.
Page 260, line 9 from bottom, for "Vol. 5" read "p. 559."

[1] *J. Soc. Chem. Ind.*, 1911, **30**, 398.

PHENOLS.

By S. S. SADTLER.

Estimation of Phenol in Crude Carbolic Acid and Tar Oils.—A method given by J. M. Weiss[1] depends upon the determination of the sp. gr. and solidifying point of a distillation fraction of the separated tar acids. These characters for pure phenol (synthesised from benzene), o-cresol (extracted from a mixture of cresols and purified by crystallisation and fractional distillation) and a mixture of m- and p-cresol (separated by fractionation from commercial mixture) are as follows:

	Phenol	o-Cresol	Mixture of m-and p-cresol
Solidif. pt., °C.	40.5	27.75	below −10
Sp. gr. at 25°/25° C.		1.0429	1.0313
B. p. range, °C.	First drop at 179	First drop at 189	First drop at 198
	1 % at 180	19 % at 190	6 % at 200
	92 % at 181	93 % at 191	81 % at 201
	100 % at 182	100 % at 192	100 % at 202

In carrying out an estimation, 300 c.c. of the tar acids separated in the usual manner are distilled in a flask fitted with a Hempel tube until the vapour temperature is 170°. Any phenol in the aqueous distillate is separated and returned to the distilling flask, and two further fractions are collected, viz., 170°–190° and 190°–202°. The last-named is redistilled and all coming over below 197° is added to the 170°–190° fraction, which now should contain all the phenol and no homologues higher than the cresols. The solidifying point and sp. gr. of this fraction are determined, whereupon the percentage of phenol may be estimated from the following data, showing the percentages of phenol corresponding with different sp. gr. for mixtures of different solidifying points.

Phenol content of mixture	Mixtures solidifying at				
	Below 0°	0° − 5°	5° − 10°	10° − 15°	15° − 23°
%	sp. gr. at 25°/25° C.	sp. gr. at 25°/25° C.	sp. gr. at 25°/25° C.	sp. gr. at 25°/25° C.	sp. gr. at 25°/25° C.
0	1.032	1.038	1.039	1.040	1.041
5	1.035	1.040	1.041	1.042	1.403
10	1.040	1.042	1.043	1.044	1.045
20	1.043	1.045	1.047	1.048	1.048
30	1.047	1.048	1.050	1.051	1.052
40	1.049	1.052	1.053	1.054	1.055
50			1.055	1.057	1.058
60					1.061

[1] *J. Gas Lighting,* 1913, 122, 820.

For mixtures solidifying above 23°, the phenol content may be estimated from the solidifying point alone, viz., 23.5°, 70%; 29.5°, 80; 32.75°, 85; 35.5°, 90 and 37.75°, 95% of phenol. The figures given whilst not accurate for all possible mixtures of phenol and the cresols, cover satisfactorily all those likely to be obtained in practice and the results (referred to the original material) will be accurate to within 0.2 – 1.5 %, according to the proportion of tar acids present.

Effect of Temperature, Acid Concentration and Time on the Bromination of Phenol in Quantitative Estimations.—Rhodes and Redman[1] have shown that if the concentration of the phenol be about $N/100$, the reaction with bromine (hypobromite solution or a bromide-bromate mixture) is complete in about 1 minute when thoroughly shaken and only tri-bromophenol is formed as a white flocculent precipitate. Experiments by Redman, Weith and Brock,[2] on the effects of varying experimental conditions on the reaction have shown that for the complete bromination of phenol in 1 minute at 22° in a solution containing free acid in a concentration of $0.8N$, a 2% excess of free bromine is sufficient. It is stated that in this way phenol may be estimated to within 0.00005 grm.

The solutions used are $N/10$ sodium thiosulphate and an $N/10$ solution of either hypobromite or bromide-bromate, 20% KI and 1/2% starch solutions.

The procedure is as follows: Into a 500 c.c. bottle, fitted with a ground glass stopper, put 60 c.c. water, 5 c.c. hydrochloric acid (sp. gr. 1.2) and then add 15 c.c. of the unknown phenol solution which is to be determined and which has previously been diluted to about $N/10$. If the solution is weaker than $N/10$ no previous dilution is required. Add quickly enough $N/10$ hypobromite or bromide-bromate solution to make the solution yellow and then add in addition 10% of the amount already added. Place the stopper in the bottle and shake continuously for 1 minute. Add to the solution in the bottle 5 c.c. potassium iodide solution (10%) and again shake for 3 minutes. Wash down the stopper and sides of the bottle and titrate the solution with the $N/10$ thiosulphate, using starch solution as an indicator. The starch must not be added until enough thiosulphate has been run in to make the solution almost colourless. The quantity of thiosulphate used represents the quantity of free iodine and therefore the quantity of excess bromine. The difference between this quantity and the known quantity of bromine added gives the amount of solution present. Each c.c. of $N/10$ bromine used up is equivalent to 0.00156 grm. of phenol.

The Value of the Higher Phenols in Wood-preserving Oils—2 grm. each of heavy creosote oil and of phenols extracted from the same were exposed by Cabot[3] on a watch glass at a temperature of from 50°–55° for 200 hours and the loss of weight noted at stated intervals. The residue

[1] *J. Ind. Chem.*, 1912, 4, 655.
[2] *J. Ind. Eng. Chem.*, 1913, 5, 389–393.
[3] *J. Ind. Eng. Chem.*, 4, 206, 1912.

of oil left on the watch glass was a viscous fluid. That of the tar acids had a pitch-like consistency at 15°. The following tables are taken from many giving similar results.

No. 2 tar acids		No. 1 heavy creosote oil	
Time, hours	Loss, %	Time, hours	Loss, %
1	1.35	1	2.1
4	3.5	4	6.35
7	7.6	7	9.5
12	8.8	12	10.0
15	10.2	15	11.25
18	10.5	18	12.1
43	15.6	43	16.5
67	18.8	67	22.2
94	20.8	94	25.5
140	23.0	140	32.1
164	24.3	164	34.3
188	25.3	188	36.6
200	25.75	200	36.3

The tar acid residue in these experiments when boiled with a 35% solution of sodium hydroxide was apparently insoluble. It was also insoluble in a 25% solution. Another portion of the tar acid residue was then dissolved in 90% of its weight of benzene and extracted with a warm 10% solution of sodium hydroxide three times. The benzene assumed a dark translucent colour, the alkali a clear brown and a black tarry layer remained between, after settling over night. The alkaline extract was washed free from tarry matter with benzene, neutralised with sulphuric acid and extracted with ether in the usual way. This extract was found to contain less than 10% of the original tar acids dissolved in the benzene. It was found that the tarry layer was partially soluble in water, while the remainder after washing with water redissolved in benzene, showing that it had been dragged down only mechanically.

Behaviour of Phenols, Naphthols and Phenolcarboxylic Acids towards Tetravalent Titanium.—According to O. Hauser and A. Lewite[1] phenols, naphthols and naphtholsulphonates give with concentrated solutions of titanium dioxide in hydrochloric or sulphuric acid a deep red or violet colour, affording a general test for hydroxyl-groups. Compounds containing two adjacent hydroxyl-groups give the test in quite dilute solution. Halogens and nitrogen prevent the appearance of the colour, but other organic radicals do not, so long as the hydroxyl-group is left free. It was not possible to isolate the compounds in well-defined form; their stability seems to increase with the number of hydroxyl groups. Carboxyl groups increase their stability still further. Thus titanium dioxide and an excess of salicylic acid (more than 3 molecules) in hydrochloric acid, slowly treated with ammonia until the solution is just faintly acid, give tetra-ammonium dititansalicylate $O:Ti_2$ $(OC_6H_4 \cdot CO_2)_6[(NH_4)_4H_2],2H_2O$, in golden yellow prisms, easily hydrolysed by water, ammonia, alkalis and mineral acids. The disodium salt forms golden yellow leaflets with $4H_2O$.

Cresols.—Experiments are described by C. M. Pence[2] showing that *o*- and *p*-cresol cannot be estimated by a bromine method similar to that used

[1] *Ber.*, 1912, **45**, 2480.
[2] *J. Ind. Eng. Chem.*, 1912, **4**, 518.

for the estimation of phenol. *m*-Cresol can, however, be determined in this way. For example, using 20 c.c. of *m*-cresol, 50 c.c. of $N/10$ bromine and 5 c.c. of concentrated hydrochloric acid, allowing to stand for $1\frac{1}{2}$ hours, then adding 10 c.c. of a 20% solution of potassium iodide and allowing to stand for 1-2 hours, all the *m*-cresol is converted into tribomo-*m*-cresol; if 175 c.c. of water be added, the bromination is complete in 30 minutes. When iodine acts upon *o*- and *p*-cresols di-iodo-compounds are formed and this action is used as the basis of the following method of estimating these two isomerides. 2-2.5 grm. of the cresol are dissolved in water to which 10 c.c. of $N/2$ sodium hydroxide have been added, and the solution is diluted to 1 litre. 25 c.c. of the solution are treated in a stoppered vessel with 9 grm. of sodium acetate and 50 c.c. of $N/10$ iodine solution, and after standing 1 hour, the solution is diluted with 100-200 c.c. of water, a few c.c. of chloroform added to dissolve the precipitated di-iodocresol and the excess of iodine titrated with $N/10$ thiosulphate: 1 c.c. of $N/10$ iodine is equivalent to 0.002681 grm. of cresol. *m*-Cresol does not yield a di-iodo-compound under these conditions; hence the method cannot be applied to mixtures containing *m*-cresol.

Estimation of m-Cresol in Cresol Mixtures. Raschig's Method.[1]—Exactly 10 grm. of the cresol mixture are weighed into a small conical flask, mixed with 15 c.c. of ordinary sulphuric acid of sp. gr. 1.84, then heated for 1 hour in a steam oven and the contents poured into a wide-necked flask of 1,000 c.c. capacity. The flask is cooled under the tap, shaking it round meanwhile in such a manner that the sulphonic acid, which is a mobile liquid, whilst hot, settles as a thick syrup on the sides of the flask during cooling. 90 c.c. of nitric acid of sp. gr. 1.38 are then first poured into the small flask in which the sulphonation was conducted, in order to remove any sulphonic acid adhering to its sides, rinsed well round, and then poured, all at once, into the large flask. The contents of the latter are well shaken immediately, so that all the sulphonic acid is dissolved, which takes about 20 seconds, and the flask is then placed in a draught-cupboard. After 1 minute a violent reaction occurs, red fumes are evolved, and the liquid boils; then it suddenly becomes turbid, oily drops of trinitrocresol form and collect on the bottom of the flask and after 5 minutes the action is apparently ended. The whole is allowed to stand for at least another 5 minutes, then poured into a dish containing 40 c.c. of water and the flask rinsed out with a further 40 c.c. of water into the same dish. On mixing with the water the trinitro-*m*-cresol solidifies, with liberation of nitrous fumes, to a crystalline magma. It is allowed to stand for at least 2 hours whilst the liquid cools, is then crushed with a pestle and filtered on the pump through a filter which has been tared against another one. The crystals of trinitrocresol are washed with 100 c.c. of water, dried at 95° to 100°, and weighed. If these instructions are care-

[1] *Zeit. angew. Chem.*, 1900, 759.

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fully followed 1.74 grm. of trinitro-*m*-cresol are obtained for each 1.0 grm. of *m*-cresol present in the mixture, whatever the composition of the latter.   The presence of even 10% of phenol does not diminish the accuracy, as the picric acid which is formed remains in solution; but the method must not be applied to mixtures containing very large amounts of phenol, which, however, do not often occur in practice.   In such samples the presence of phenol is detected by the boiling point, and also by the fact that the nitro-compound does not remain solid in the steam-oven at 95° to 100°, but melts, or at any rate, forms a soft paste.   Xylenols, which sometimes occur in commercial cresols, behave in a similar manner; the nitro-compound either liquefies when warm or refuses to set in the cold.   But a cresol which distils for the most part between 190° and 200°, and, therefore, contains scarcely any phenol or xylenol always yields a pale yellow crystalline mass, the weight of which when divided by 1.74 gives the weight of *m*-cresol in the mixture, the error being within 1%.   The amount of nitric acid used is considerably more than is needed for the nitration and oxidation and good results can be obtained with average cresols containing 35 to 60% of *m*-cresol, when only 70 c.c. of nitric acid are used; but if this is done the action often occurs so suddenly that there is scarcely time to mix the sulphonic acid with the nitric acid and place the flask on one side; explosions have even been known to occur under these conditions.   The recognised quantity of 90 c.c. of acid is, therefore, adhered to, and is poured, all at once, into the flask as quickly as possible, a flask having a very wide neck being used.

F. Russig and G. Fortmann[1] have described a method which is used in France.   This is not so simple or rapid as Raschig's method, but gives rather higher results, probably because the nitration is more complete; it is also inapplicable to mixtures containing more than 10% of phenol or xylenols. 50 grm. of the cresol are weighed into a small conical flask and mixed with 125 grm. of sulphuric acid of sp. gr. 1.84.   The temperature of the mixture rises spontaneously to between 60° and 70°, and further warming is unnecessary.   After standing for 1–2 hours, the sulphonic acid is nitrated in a tubulated retort of 1 litre capacity, placed on a sand-bath; the neck of the retort is connected to a wash bottle and this, in turn, to a good draught. 400 c.c. of nitric acid of sp. gr. 1.38 are placed in the retort, heated to 60°, and the flame then removed.   A cylindrical dropping-funnel without a neck is fixed in the tubulure of the retort by a rubber stopper and the small conical flask in which the sulphonation was effected is placed upside-down over the funnel in such a way that the contents are only delivered slowly and are similarly emptied from below.   The sulphonic acid is allowed to gradually drop into the hot nitric acid during a period of 1½–2 hours; it is thus completely nitrated and oxidised; the oxidation is accompanied by a violent development of heat and evolution of nitrous fumes.   About

[1] *Z. angew. Chem.*, 1901, 14, 157.

20 minutes after the conclusion of the action, the contents of the retort are poured into a dish containing 200 c.c. of water and the retort is rinsed out with a further 200 c.c. After standing over night, the crystalline mass is crushed in the dish, filtered on a hardened filter on the pump, washed with a further 200 c.c. of water, and weighed. When treated in this manner 50 grm. of pure *m*-cresol yield 87.8 grm. of trinitro-*m*-cresol, being 175.6 %, whereas by Raschig's method only 174.0% is obtained on the weight of the original cresol.

Creosote.

The following methods of the New York Testing Laboratory are recommended by the writer for creosote oils.

Sp. Gr.—By pyknometer at 38° compared with water at the same temperature.

Sulphonation.—10 c.c. of the total distillate up to 315° are placed in a flask and warmed with four to five volumes of concentrated sulphuric acid to 60° and the whole transferred to a graduated separating funnel. The flask is rinsed three times with small quantities of concentrated sulphuric acid and the rinsings added to the contents of the funnel, which is then stoppered and shaken, cautiousy at first, afterwards vigorously, for at least 15 minutes and allowed to stand over night. The acid is then carefully drawn down into the graduated portion of the funnel to within 2 c.c. of where the unsulphonated residue shows. If no unsulphonated residue shows, the acid should be drawn down to 2 c.c. In either case the test should be carried further as follows: Add about 20 c.c. of water and allow to stand $\frac{1}{2}$ hour. Then draw off the water as close as possible without drawing off any supernatant oil or emulsion; add 10 c.c. of concentrated sulphuric acid and allow to stand for 15–20 minutes. Any unsulphonated residue will now separate out clear and give a distinct reading. If under $\frac{2}{10}$ of a c.c. it should be drawn down into the narrow part of the funnel to just above the stopcock where it can be estimated to $\frac{1}{100}$ of a c.c. (0.01 c.c.). The volume of residue thus obtained is calculated on the original oil.

Tar Acids.—50 c.c. of the distillate to 315° to which 40 c.c. of a solution of sodium hydroxide (19%) having a sp. gr. of 1.15, is added, is warmed slightly and placed in a separating funnel. The mixture is vigorously shaken, allowed to stand until the oil and soda solutions separate and the soda solution containing most of the tar acids drawn off. A second and third extraction is then made in the same manner, using 30 and 20 c.c. of the sodium hydroxide solution respectively. The three extracts are united in a 200 c.c. graduated cylinder and acidified with dilute sulphuric acid. The mixture is then allowed to cool and the volume of the tar acids noted. The results shown should be calculated on the original oil.

Distillation.—100 grm. of the oil are distilled in an 8-ounce glass retort. The lower end of the bulb of the thermometer is placed $\frac{1}{2}$ in. above the

surface of the oil and the distillation conducted at the rate of 1 to 2 drops per second.

Coke Test.—In making the coke determination hard glass bulbs are to be used.

Warm the bulb slightly to drive off all moisture, cool in a desiccator and weigh. Again heat the bulb by placing it momentarily in an open Bunsen flame, place the tubulure underneath the surface of the oil to be tested and allow the bulb to cool until sufficient oil is sucked in to fill the bulb about two-thirds full. Any globules of oil sticking to the inside of the tubulure should be drawn into the bulb by shaking or expelled by slightly heating it and the outer surface should be carefully wiped off and the bulb reweighed. This procedure will give about 1 grm. of oil. Cut a strip of thin asbestos paper about 1 in.$\times\frac{1}{4}$ in., place it around the neck of the bulb and catch the two free ends close up to the neck with a pair of crucible tongs. The oil should then be distilled off, as in making an ordinary oil distillation, starting with a very low flame and conducting the distillation as fast as can be maintained without spurting. When the oil ceases to come off, the heat should be increased until the highest temperature of the Bunsen flame is reached, the whole bulb being heated red hot until evolution of gas ceases and any carbon adhering to the outside of the tubulure is completely burned off. The bulb should then be cooled in a desiccator and weighed and the percentage of coke residue calculated to water free oil.

Coal-tar creosote oils have no greater coke than 3 %.
Water-gas creosote oils have more coke than 3 %.

Antiseptic Properties of Creosote (K. V. Kharichkov[1]).—After removing the phenols, bases and unsaturated hydrocarbons from creosote by the usual methods, its antiseptic properties were tested on cultures of *Merulius lacrimans*, *Penicillum glaucum* and a parasitic fungus from decaying strawberries. The results show that the removal of the above constituents hardly influences the antiseptic properties of creosote.

Antiseptic Tests of Wood-preserving Oils.—A. L. Dean and C. R. Downs[2] have made experiments with *Polystictus versicolour*, which was obtained in pure culture from decaying wood. This is the fungus that destroys 75% of the broadleaf timber used for ties. The culture medium was prepared by adding 0.5% cane sugar and 0.5% asparagine to germinated bean extract and then stiffened by adding 1.5% agar-agar. The creosote oils were emulsified by grinding with an equal weight of gum arabic, adding water from time to time. A small piece of the medium to be inoculated was cut out and laid to one side, the transferred mycelium and agar from the stock culture placed in the cavity and the piece of creosoted medium replaced on top. In this way the mycelium was buried and if it grew up through and vegetated on the surface, there could be no question that the antiseptic was

[1] *J. Russ. Phys. Chem. Soc.*, 44, 345–348.
[2] *Orig. Com.*, *8th Intern. Congr. Appl. Chem.*, 13, 103–210.

insufficient. The results showed that coal-tar creosote is a stronger antiseptic than water-gas tar creosote, and that the latter is more effective than the liquid oils of the anthracene fraction of coal tar. The greater value of the coal-tar creosote appears to depend upon the presence of the tar acids and especially upon the tar bases. The water-gas tar creosote was almost identical in antiseptic power with the coal-tar oil which had had its acids removed. Allman's work indicated that the oils remaining in wood treated with coal-tar creosote were almost free from tar acids after a few years and that the lighter hydrocarbons may all disappear. Loss of antiseptic power from disappearance of tar acids cannot take place with water-gas tar oils, since they are free from phenols from the beginning. Since the amount commonly injected into wood is 10 pounds or more per cubic foot, the difference in antiseptic power between coal-tar oils and water-gas tar oils is not of great significance.

Note on the Rideal-Walker Phenol Control.—A new method is presented by Walker and Weiss,[1] of insuring the purity of phenol used in determining the bactericidal efficiency of disinfectants and to show that the presence of cresols probably accounts for the varying results obtained. Different mixtures of cresol in 0–30% strength with synthetic phenol were made and the lowering of the melting point was found to be constant, for any particular degree of this impurity, regardless of the varying proportions of the three isomeric cresols. A curve plotted showed the solidifying point to be a linear function of the per cent. of phenol up to at least 30% cresol. From this if the solidifying point is known, the degree of cresol contamination can be determined. The bactericidal efficiency of cresol is three times that of phenol. The various estimations made with synthetic phenol agree closely with the calculated theoretical figures. It is well known that phenol crystals are usually contaminated by cresols to such an extent as to make them unreliable for purposes of bactericidal control. This impurity depresses the coefficient of the disinfectant. The bromine titration is insufficient to insure the purity of the phenol. The solidifying point is the best test. No phenol showing a solidifying point of less than 40° should be used for purposes of bactericidal control.

Detection of Natural Asphaltum and Petroleum Pitch in Residues from the Distillation of Coal Tar.[1]—A method of detecting natural asphaltum or petroleum pitch in the residues from the distillation of coal tar is based upon the fact that distillates of the latter (consisting in the main of aromatic hydrocarbons) are converted by sulphuric acid almost quantitatively into sulphonic acids which are soluble in water, whereas the distillates from natural asphaltum or petroleum pitch are only affected to a limited extent by this treatment. If, however, large quantities (e.g., up to 20%) of these bituminous products are present in coal-tar pitch, the distillation causes decomposition, with the formation of unsaturated hydrocarbons capable of being attacked by sulphuric acid. The following method of separating the compounds

[1] J. Frank. Inst., 174, 101–12.
[2] F. Schwarz, Chem. Rev. Fett. Ind., 1913, 20, 28–30.

which combine with sulphuric acid obviates the necessity of distillation: 10 grm. of the pitch are heated to 160°–180° in an oil bath, then stirred for 5 minutes with 4 c.c. of concentrated sulphuric acid and the temperature kept at 180° until all sulphurous acid and excess of sulphuric acid have been expelled. The mass is then thoroughly ground up with 40 grm. of bone charcoal, and extracted in a Soxhlet apparatus with petroleum spirit of low boiling point, the extract evaporated, the residue taken up with more petroleum spirit, the solution filtered and evaporated and the residue weighed. Any sulphur present is removed by treating the residue (without stirring) with acetone, filtering the solution, evaporating the filtrate and again weighing the residue. The amounts of constituents not attacked by sulphuric acid varied as follows in the case of the samples examined: coal-tar pitches, 0.10–0.20; natural asphaltums, 1–16 (usually 3–8); and petroleum pitches, 6–36% (usually 15–30%). If the result obtained as above greatly exceeds 0.2%, the presence of natural asphaltum or petroleum pitch is indicated.

Application of the Dimethyl Sulphate Test for Detecting Small Amounts of Petroleum or Asphalt Products in Tars.[1]—The material is not distilled to coke and a test made on the total distillate, as in the Sommer method, but into three fractions: 270–315°, 315–350°, and 350–375°. The asphalt distillate concentrates in these fractions, especially the last, and can easily be detected by taking 4 c.c. of the fraction, mixing with 6 c.c. dimethyl sulphate in a 10 c.c. cylinder graduated to 0.2 c.c. and shaking. After standing, the layer of insoluble petroleum can be read. The method does not give quantitative results, but when the grades of asphalt and tar oil in a mixture are known, tests on like laboratory mixtures enables one to determine within narrow limits the per cent. of each constituent in the material under investigation.

<center>ERRATA IN VOL. III.</center>

Page 263, line 11, for "dihydoxyanthraquinone" read "dihydroxyanthraquinone."

Page 273, line 14 from bottom, for Vol. 5 read Vol. 6.

Page 278, line 15, for "naphthalquinone" read " naphthaquinone." Line 18, insert brackets round "diphenyleneketone."

Page 293, line 17 from bottom, for "absoption" read "absorption."

Page 301, line 16 from bottom, for "Nortmann" read "Vortmann."

Page 308, line 5, insert comma after "sample." Line 7 from bottom, for "theire" read "their."

Page 314, line 9 from bottom, for "0.7379" read "0.7397." Line 4 from bottom for "with" read "within."

Pagees 319 and 320, in several places, for "pyridin" read "pyridine."

Page 321, line 3 from bottom, for "chlorid" read "chloride."·

Page 324, line 15, for "page 160" read "page 25."

Page 345, line 20, for "Gassetta" read "Gazzetta."

Page 348, line 5 from bottom, for "20°" read "−20°."

Page 364, line 4, in table, for "105" read "195."

Page 383, line 21 from bottom, for "dimethynaphthalenes" read "dimethylnaphthalenes."

Page 390, heading of Table, for "Beaumé" read "Baumé."

[1] Charles S. Reeve and Richard H. Lewis, *J. Ind. Eng. Chem.*, 5, 293–295.

AROMATIC ACIDS.

By EDWARD HORTON.

Phenol-p-sulphonic Acid.

Estimation.—The following volumetric method is described by Smith and Frey.[1] A quantity of the sample corresponding with 0.18–0.20 grm. of phenol-p-sulphonic acid is dissolved in 50 c.c. of water in a stoppered long-necked 250 c.c. flask. To this are added 50 c.c. of an aqueous solution containing 2.7833 grm. of potassium bromate and about 40 grm. of potassium bromide per litre. 5 c.c. of hydrochloric acid (D 1.18) are then added, the flask stoppered to prevent loss of bromine and the mixture kept at 20°–25° for not less than 10 and not more than 15 minutes, during which time no turbidity should be produced. Dibromophenolsulphonic acid is formed by the action of the free bromine, the excess of which is determined by titration with standard thiosulphate solution after the addition of potassium iodide. The method is accurate to $\pm$ 0.5%.

Benzoic Acid and Its Derivatives.

Commercial Benzoic Acid.—For the detection of halogens in the benzoic acid of commerce, Wende[2] gives the following test: A mixture of 0.1 grm. of the sample with 0.5 grm. of yellow mercuric oxide is heated in a dry test-tube, the latter being constantly turned over a flame about 1 cm. high. When the action is over, about 10 c.c. of dilute nitric acid are added, the whole heated nearly to boiling and filtered. The filtrate should not give more than a slight opalescence with silver nitrate solution.

The British Pharmacopœia 1914 requires benzoic acid to conform to the following standard:

"When 0.5 grm. is heated in a closed crucible with twice its weight of calcium carbonate, the mass dissolved in diluted nitric acid, and solution of silver nitrate added, not more than the slightest cloudiness results (absence of chlorobenzoic acid). Yields no characteristic reactions for oxalates. Does not develop the odour of benzaldehyde when warmed with its own weight of potassium permanganate and 10 times its weight of dilute sulphuric acid (absence of cinnamic acid). Arsenic limit 2 parts per million."

Reactions and Detection.—Since the publication of Vol. III several authors have published new methods for the detection of benzoic acid, or

[1] *J. Amer. Chem. Soc.*, 1912, **34**, 1040.
[2] *Apoth. Zeit.*, 1914, **29**, 157.

modifications of old ones. The test devised by Mlle. Anna Jonescu seems
to meet with considerable commendation, being applied or recommended
by Halphen, Marchadier, Thomann, Denigès, Biernath, Philippe and
Fleury. On the other hand, Fischer and Gruenert state that it is far inferior
to the modified Mohler method.

Fleury[1] points out that the Jonescu reaction takes place very slowly in
cold solutions, whilst heat is liable to carry it too far and so to cause failure.
He hastens the action by the addition of a trace of ferrous sulphate to act as
catalyst. 10 c.c. of the solution to be tested (containing 1–5 mg. of benzoic
acid) are treated with 3 drops of a solution of ferric chloride (sp. gr. 1.260,
containing about 26% of the anhydrous salt) diluted 1 to 10, then with 3
drops of a solution of hydrogen peroxide (12 vol.) also diluted 1 to 10, and
finally with 3 drops of 3% solution of ferrous sulphate. The reagents should
be added in the order named, shaking after each addition. In about 30
seconds action commences, and the violet colouration attains its maximum
in 5–10 minutes. The test is sensitive to 0.0002 grm. of benzoic acid.

Denigès[2] publishes the following modification of Jonescu's method. To
4 c.c. of an aqueous solution supposed to contain benzoic acid, 0.2 c.c. of
20% (by volume) acetic acid, 0.2 c.c. of ferric chloride solution (dilute) and
0.2 c.c. of hydrogen peroxide solution (one volume) are added and the
mixture is boiled for 10–15 seconds. A violet colouration is obtained
even when the solution contains not more than 0.0005 grm. of benzoic acid
per 1 c.c. In cases where smaller quantities are to be detected a blank
experiment should be made simultaneously, both test-tubes being heated
in boiling water for the same length of time.

Halphen[3] points out that if in preparing the solution for the Jonescu test
ammonia is used to neutralise the acid before evaporating to dryness, 0.001
grm. of benzoic acid will fail to give the test, whilst by using sodium hydroxide
a sharp indication is obtained.

von der Heide and Jakob[4] have described the following method to detect
benzoic acid in wine. A sample of the wine is made alkaline and evaporated
until all the alcohol has been driven off. The residual solution is acidified,
submitted to steam distillation, and the distillate extracted with ether. The
ethereal solution is evaporated and the residue tested by Robin's modifica-
tion of Mohler's method (Vol. III, p. 410). The method is stated by
Polenske[5] to be trustworthy.

For the detection of benzoic acid in milk Revis[6] has devised the following
process. At least 100 c.c. of the milk are diluted with an equal volume of
water, 5 c.c. of a 10% solution of sodium carbonate are added, and the mix-

[1] J. Pharm. Chim., 1913 [vii], 8, 460.
[2] Bull. Soc. Pharm. de Bord., 1911, 249; Pharm. J., 1911, 87, 201.
[3] Matières grasses., 1910, 3, 1761.
[4] Zeitsch. Unter. Nahr. Genussm., 1910, 19, 137.
[5] Arbeit. Kaiserl. Gesundheitsamte, 1911; 38, 149.
[6] Analyst, 1912, 37, 346.

ture is heated in boiling water for 2–3 minutes. 10 c.c. of a 20% solution of calcium chloride are added, the heating is continued until the casein is completely coagulated, the liquid cooled and filtered, and the filtrate made neutral to litmus paper with hydrochloric acid. To the neutral filtrate 10 c.c. of copper sulphate solution (as made for Fehling's solution) and 10 c.c. of potassium hydroxide solution (containing 31.18 grm. per litre) are successively added and the liquid again filtered. The filtrate is transferred to a separating funnel, acidified with hydrochloric acid and shaken with 50 c.c. of ether. The aqueous solution is run off, the ether washed three times with a little water, 10 c.c. of water and a drop of phenolphthaleïn solution are added, and a saturated solution of barium hydroxide is run in gradually, with shaking, until a permanent pink colour is produced. The aqueous layer is now filtered into a porcelain basin, evaporated to about 5 c.c., filtered into a test-tube and 1% acetic acid added until the liquid is decolourised. Two more drops of the acetic acid are then added, and 1 drop of a freshly prepared neutral solution of ferric chloride (10%). With as little as 0.02% of benzoic acid the characteristic precipitate of ferric benzoate is obtained. In examining cream 50 c.c. are diluted with water to 200 c.c. and then treated as above.

Philippe[1] extracts the benzoic acid from the milk by a similar method and identifies it by Robin's or Jonescu's test.

According to Hinks[2] 0.01% or more benzoic acid in milk or cream can be detected as follows: 25 c.c. of the milk or from 10 to 20 grm. of the cream are heated with an equal volume of concentrated hydrochloric acid until the curd has completely dissolved. The liquid is cooled, shaken with 25 c.c. of a mixture of light petroleum (2 vols.) and ether (1 vol.), the ethereal solution separated and shaken with 1 drop of ammonia and 5 c.c. of water. The aqueous layer is now separated, heated on a water-bath for a few minutes to expel the excess of ammonia and then tested with ferric chloride solution. On adding the ammonia, ammonium benzoate is precipitated and this test although less characteristic is more sensitive than the ferric chloride test.

Fischer and Gruenert[3] have continued their work on the detection of benzoic acid in meat and fats. They find that the methods of Halphen and Robin which are applicable to butter are entirely inapplicable to meats and other fats. On the other hand, von der Heide and Jakob's modification of Mohler's method gave a sharp indication with as little as 0.01% of benzoic acid in 50 grm. of substance. The authors prefer to extract the preservative from meats by the method previously described (Vol. III, p. 411), but for butter and margarine they recommend the following process: 50 grm. of butter are introduced into a 300 c.c. Erlenmeyer flask, treated with 150 c.c. of 1% sodium hydrogen carbonate solution and boiled for 5 minutes with

[1] *Mitt. Lebensmittel-unters. Hyg.*, 1911, 2, 377.
[2] *Analyst*, 1913, 38, 555.
[3] *Zeitsch. Nahr. Genussm.*, 1912, 20, 580.

frequent shaking If necessary a blast of air is used from time to time to prevent frothing. The hot liquid is transferred to a separating funnel and the aqueous layer drawn off, neutralised with $N/2$ sulphuric acid solution, using methyl-orange as indicator, and clarified by adding 10 c.c. of Fehling's copper sulphate solution and 10 c.c. of potassium hydroxide solution (containing 31.15 grm. per litre). The liquid, which should be neutral or faintly acid, is filtered, the filtrate acidified with dilute sulphuric acid and extracted with ether. The ethereal extract is washed, evaporated and the residue tested by von der Heide and Jakob's method.

Robin states[1] in reference to his modification of Mohler's method that since salicylic acid and other phenolic derivatives respond to the test, these must be removed by dissolving the benzoic acid extract in dilute sulphuric acid, adding 10% potassium permanganate solution until the red colouration becomes permanent after heating at 80°, and then extracting with ether and proceeding with the test (Vol. III, p. 410).

For the detection of benzoic acid in butter, methods are described by Marchadier[2] and Biernath[3] in which the sample is distilled with water, a little sulphuric acid and a small quantity of pumice-stone and the distillate tested by Jonescu's method. Biernath states that the reaction is retarded by mineral acids, volatile organic acids and alcohol.

Friese[4] and Volhase[5] have described other methods of detecting benzoic acid in fats, but these are similar to that of Fischer and Gruenert.

Lythgoe and Marsh[6] have observed that when the ethereal extract of coffee or pure coffee extract is treated with ferric chloride solution in the usual manner for the detection of benzoic acid, a precipitate is obtained which differs from ferric benzoate in colour, and yields crystals different from those of benzoic acid on sublimation. The ammonium salt of the substance which produces this precipitate is distinguished from ammonium benzoate in that it gives precipitates with manganese, nickel, magnesium, calcium, barium and strontium. Accordingly when looking for benzoic acid in coffee extract these authors recommend that the acid solution should be extracted several times with ether, the ether extract washed with water and extracted with ammonia. The ammoniacal solution is evaporated to a small volume, adding ammonia to prevent development of acidity, and treated with a solution of manganese sulphate. It is then filtered and the filtrate tested for benzoic acid with ferric chloride solution.

A new test for benzoic acid has been discovered by Schmatolla.[7] If 20 c.c. of a solution containing benzoic acid are treated with 5 c.c. of hydrogen peroxide solution, and then with freshly prepared solution of 5 grm. of ferrous

[1] Ann. Falsif., 1913. 6, 277.
[2] Ann. Falsif., 1911, 4, 28.
[3] Apoth. Zeit., 1912, 27, 192.
[4] Pharm. Zentr., 1911, 52, 1201.
[5] Chem. Zeit., 1913, 37, 312.
[6] J. Ind. Eng. Chem., 1911, 3, 842.
[7] Pharm. Zeit., 1913, 57, 947.

sulphate and 5 grm. of boric acid in 100 c.c. of water, a blue or greenish-blue colour appears in a few seconds.

Estimation.

Volumetric Methods.—An iodometric method of estimating benzoic acid has been described by Remy.[1] The acid (0.05–0.5 grm.) is dissolved in 30 c.c. of 50% alcohol. 5–10 c.c. of 5% potassium iodide solution and an equal volume of 5% potassium iodate solution are added and the liberated iodine titrated with thiosulphate solution. The benzoic acid is converted into mono-iodobenzoic acid, and one molecule of benzoic acid is equivalent to one atom of iodine.

For the estimation of benzoic acid in benzoates Lyons[2] dissolves 0.25 grm. of the salt in 10 c.c. of water in a separating funnel, acidifies with 25 c.c. of $N/10$ sulphuric acid solution and extracts four times with chloroform (which must be neutral). The chloroform is run into a second separating funnel, washed with 20 c.c. of water, transferred to a flask and titrated with $N/25$ alkali solution using methyl-red as indicator. The end point of the titration is indicated by the appearance of a yellow colour in the aqueous layer after shaking with the chloroform.

Whilst extraction with chloroform can be applied to the estimation of benzoic acid in cranberries, Folin and Flanders[3] found that certain precautions have to be taken in the case of ketchups, from which chloroform extracts other acids also. 25 grm. of the ketchup are placed in a 50 c.c. beaker, treated with 2 c.c. of concentrated nitric acid and about 0.2–0.3 grm. of sodium nitrite added in small portions, the mixture being well stirred after each addition. The liquid is rinsed into a 500 c.c. separating funnel with the help of 200 c.c. of saturated ammonium sulphate solution, and is then extracted five times with chloroform (50 c.c., 35 c.c., 25 c.c., 25 c.c., 25 c.c.). The chloroform is run into another separating funnel and shaken with 200 c.c. of a saturated solution of sodium chloride made faintly acid with hydrochloric acid. It is then transferred to a third separating funnel, again shaken with 200 c.c. of the acidified salt solution, and finally run into a 500 c.c. Erlenmeyer flask and titrated with standard alcoholic sodium ethoxide solution in the presence of phenolphthalein.

Cinnamic acid is not removed by the washing with salt solution, hence any present will be estimated with the benzoic acid. The sodium ethoxide solution is prepared by dissolving 2–3 grm. of sodium in a litre of absolute alcohol; it is standardised against benzoic acid in chloroform solution.

Raiziss and Dubin[4] sought for a solvent which would not form emulsions with urine. They found that toluene is preferable to the chloroform recom-

[1] *Apoth. Zeit.*, 1911, 26, 835.
[2] *J. Amer. Pharm. Assoc.*, 1912, 1, 526.
[3] *J. Amer. Chem. Soc.*, 1911, 33, 1622.
[4] *J. Biol. Chem.*, 1915, 20, 125.

mended by Folin and Flanders. 100 c.c. of fresh urine are acidified with 1 c.c. of concentrated nitric acid, then saturated with ammonium sulphate (50–60 grm. being required) and extracted four times with toluene (50 c.c., 40 c.c., 30 c.c., and 30 c.c.). The toluene extracts are mixed and washed with 100 c.c. of saturated salt solution containing 0.05% of concentrated hydrochloric acid. The washing is repeated and then the benzoic acid in the toluene solution is titrated with $N/20$ sodium ethoxide solution using phenolphthalein as indicator. The ethoxide solution is prepared by dissolving 2.3 grm. of sodium in alcohol and diluting the solution to 2000 c.c. Hippuric acid is not extracted by toluene under the above conditions.

McAbee[1] has applied the provisional A. O. A. C. method of estimating sodium benzoate to ketchups containing added known weights of the salt and has shown that the method is reliable.

To estimate salicylic and benzoic acids and saccharin in fruit juices, jams, and lemonades, van Raalte recommends[2] boiling these substances for 5 hours with dichloroethylene followed by titration with $N/10$ alkali solution and phenolphthalein. Then the neutral aqueous liquid, after separation from the dichloroethylene, is examined for the two acids and saccharin. The latter can be extracted with ether after addition of phosphoric acid.

In a report[3] on cooperative work on the determination of sodium benzoate in jams, jellies, and salt codfish, Dunbar advocates making the former two substances alkaline with milk of lime instead of sodium hydroxide, the latter being used with codfish. The sodium benzoate is then extracted with a definite volume of water, of which an aliquot part is saturated with sodium chloride, filtered, acidified and extracted with chloroform, the chloroform solution being evaporated and the residue titrated.

Van der Laan and Tijdens[4] recommend extracting benzoic acid from foods by means of benzene for quantitative estimation.

Polenske[5] has described a method of estimating benzoic acid in cranberries.

Gravimetric Methods.—Hinks[6] states that the benzoic acid present in milk may be estimated by heating 25 c.c. with hydrochloric acid in a reflux apparatus, extracting the cooled solution three times with 20 c.c. of a mixture of ether (1 vol.) and light petroleum (2 vols.) and shaking the separated ethereal solutions with 10 c.c. of water and 1 drop of ammonia; this extraction is twice repeated. The mixed aqueous portions are then acidified with hydrochloric acid, extracted three times with the ethereal solvent, the extracts evaporated at ordinary temperature and the residue dried in a desiccator to constant weight. The benzoic acid is then volatilised at 100° and its amount determined by the loss in weight.

[1] *J. Ind. Eng. Chem.*, 1910, 2, 544.
[2] *Chem. Weekblad.*, 1912, 9, 1004.
[3] *U. S. Dept. Agr. Bur. Chem.*, Bull. 137, 108.
[4] *Chem. Weekblad.*, 1910, 7, 603.
[5] *Arbeit. Kaiserl. Gesundheitsamte*, 1911, 38, 149.
[6] *Analyst*, 1913, 38, 555.

Liverseege and Evers[1] have described an empirical method of estimating benzoic acid in milk, by which, however, only about 45% of the acid is obtained.

Hillyer[2] has devised a method of estimating benzoic acid in ketchups depending on the precipitation of the acid from alcoholic solution as silver benzoate and weighing as such. To estimate benzoic acid in chopped meat Krüger[3] recommends the following out of several modifications of the distillation method. 50 grm. of the chopped meat are mixed with enough sulphuric acid, making allowance for the moisture content of the sample, to have present 45 c.c. of 70% sulphuric acid. The mixture is heated until clear and then steam distilled (keeping the volume constant) until 500 c.c. of distillate have been collected. The cold distillate is filtered and the filter washed, the filtrate made faintly alkaline with sodium hydroxide solution and evaporated to small bulk on a water-bath. To remove impurities, a cold saturated solution of potassium permanganate is added until the red colour produced persists for 5 minutes. The excess of permanganate is destroyed with sodium sulphite solution and the liquid evaporated to 10 c.c. After cooling it is transferred to a separating funnel and acidified with dilute sulphuric acid (1:3). The manganese precipitate, remaining in the basin is dissolved in cold saturated sodium sulphite solution and sulphuric acid and transferred to the funnel. The liquid is extracted three times with an equal volume of a mixture of ether and light petroleum, the ethereal solution washed three times with 3 c.c. of water and dried with a small amount of powdered gum tragacanth. It is then allowed to evaporate spontaneously in a tared basin, dried and weighed. The results can be checked by subliming the product and reweighing or by titration. From 97 to 120% of the benzoic acid present is recovered by this method.

Metallic Benzoates.

The British Pharmacopœia 1914 fixes the following standard of purity for the sodium and ammonium salts:

Sodium Benzoate.—"Loses not more than 4% of its weight when dried at 112°. 1 grm. of this dried salt heated to redness until gases cease to be evolved leaves an alkaline residue which treated with water, filtered and well washed, yields a clear solution requiring for neutralisation not less than 13.7 c.c. nor more than 13.9 c.c. of $N/2$ solution of sulphuric acid. Yields no characteristic reactions for copper, iron, potassium or carbonates, and not more than the slightest reactions for chlorides or sulphates. Lead limit 10 parts per million. Arsenic limit 2 parts per million."

Ammonium Benzoate.—"If 1 grm. is dissolved in 20 c.c. of water and excess of nitric acid added a crystalline precipitate of benzoic acid separates, the filtrate from which remains clear on the addition of solution of barium chloride, and does not become more than slightly opalescent on the addition of solution of silver nitrate. Lead limit 10 parts per million. Arsenic limit 2 parts per million."

[1] J. Soc. Chem. Ind., 1913, 32, 319.
[2] J. Ind. Eng. Chem., 1909, 1, 538.
[3] Zeitsch. Nahr. Genussm., 1913, 26, 12.

Benzoic Aldehyde. Benzaldehyde.

Estimation. Volumetric Method.—Dodge states[1] that benzaldehyde can be estimated by the following modification of Ripper's method. 0.15 grm. of the aldehyde is mixed in a flask with 25 c.c. of $N/5$ sodium hydrogen sulphite solution and dissolved by gentle shaking. The flask is corked and kept in ice-water for 1.5 to 2 hours. The solution is then titrated cold with $N/10$ iodine solution using starch as an indicator. The aldehyde can also be estimated by allowing about 1 grm. to stand at room temperature with 10 c.c. of 2.5 N-alcoholic potassium hydroxide solution for 24 hours and then titrating the free alkali. But oil of bitter almonds cannot be assayed by this method.

Gravimetric Methods.—The method described by Denis and Dunbar[2] has been studied both by Smith and by Woodman and Davis. Smith dealt with the application of the method to maraschino products. He found[3] that distillation in an atmosphere of carbon dioxide gave somewhat better results than in air, whilst distillation from strong sodium chloride solution gave results which were too high. Also that it is best to keep the volume of the solution during precipitation as small as possible, that the results were not affected by the presence of 20% of alcohol (by volume) and that larger amounts may be removed by distillation without affecting the estimation. The precipitate was filtered on two superimposed filter papers, one of which had been counterbalanced against the other, and was again used as a counterpoise after drying. The mean amount of benzaldehyde recovered was 88%.

Woodman and Davis[4] state that to obtain accurate results by the Denis and Dunbar method with small quantities the volume of the solution should not exceed 110 c.c. and the alcohol content should be between 8 and 12% by volume. According to these authors the method is not suitable for such small quantities of benzaldehyde as are present in maraschino cherries, for which they recommend a modified method. 100 c.c. of the liquor from maraschino cherries or 50 c.c. of maraschino liqueur, are diluted to 140 c.c. and 110 c.c. distilled off. After approximately determining the alcohol content in a small portion of the distillate 100 c.c. are mixed with alcohol or water sufficient to bring the alcohol content to about 10% by volume. The solution is then shaken vigorously for 10 minutes in a rubber-stoppered flask with 100 c.c. of the freshly prepared phenylhydrazine reagent (3 c.c. of glacial acetic acid mixed well with 40 c.c. of water and 2 c.c. of phenylhydrazine and filtered through several thicknesses of filter paper). The precipitate is collected in a tared Gooch crucible, washed first with cold water and then with 10 c.c. of 10% alcohol and dried either in a vacuum desiccator at 20 cm. pressure for 20–24 hours or in a vacuum oven at 70°–80,

[1] Eighth Inter. Cong. App. Chem., Sect. VIII b. Orig. Comm. 17, 15.
[2] Vol. III, p. 419.
[3] U. S. Dept. Agr., Bur. Chem., Bull. 152, 192.
[4] J. Ind. Eng. Chem., 1912, 4, 588.

for 3 hours. The weight of precipitate obtained in a blank determination is deducted and the corrected weight multiplied by 0.5411 to obtain the weight of benzaldehyde. The method can be applied to almond extracts by using 10 c.c. of the extract diluted to 100 c.c., and 15 c.c. of the reagent. In maraschino cherries the benzaldehyde expressed in mg. per 100 c.c. of the liquor should not be more than two or three times the alcohol content expressed in percentage by volume.

According to Golse[1] the French official method of estimating hydrocyanic acid and benzaldehyde in liqueurs is untrustworthy, the chief objections being the use of insufficient alkali to fix the hydrocyanic acid and faulty conditions of precipitation of the aldehyde phenylhydrazone. The author recommends the following procedure: 200 c.c. of the liqueur are treated with 1 c.c. of sodium hydroxide solution (sp. gr. 1.32–1.36) in a 600 c.c. flask and distilled until 175 c.c. of distillate have been collected (A). The residual liquid in the flask is then cooled, 50 c.c. of 10% sulphuric acid solution are added slowly from a tap-funnel and the mixture again distilled 50 c.c. of distillate being collected in a receiver containing 5 c.c. of ammonia (B). The distillate (A) is now transferred to a distillation flask, 5 c.c. of the phenylhydrazine reagent added, the mixture diluted to 200 c.c., and 75 c.c. distilled over; this distillate contains traces of hydrocyanic acid and is added to the distillate (B). The lower end of the condenser is then closed and the flask heated on a water-bath until the hydrazone has been precipitated, 2. hours heating being usually sufficient. The precipitate is collected on a filter paper, washed with water and dissolved by treatment with 10 c.c. of alcohol followed by 20 c.c. of ether. The alcohol-ether solution is evaporated, the residue dried in *vacuo* and weighed. The weight × 2.7 gives the weight of benzaldehyde in a litre of the liqueur. The phenyl-hydrazine reagent is prepared by dissolving 1 grm. of phenylhydrazine in a mixture of 4 c.c. of glacial acetic acid, 2 grm. of sodium acetate and 20 c.c. of water, heating and shaking vigorously, adding 1 c.c. of sodium hydrogen sulphite solution and filtering. The presence of the sodium hydrogen sulphite prevents the oxidation of the phenylhydrazone during precipitation.

The distillate (B) is used for the estimation of the hydrocyanic acid.

Feinberg[2] has made an investigation of the methods of estimating typical aldehydes, and publishes results which show that for benzaldehyde the neutral sulphite method is better than that with bisulphite, and that precipitation with *p*-nitrophenylhydrazine gives better results than using *p*-bromophenylhydrazine, the neutral sulphite method being perhaps slightly less accurate than precipitation with *p*-nitrophenylhydrazine. The best results are stated to be obtained as follows: 25 c.c. of a 1% solution of the aldehyde in 12% acetic acid are diluted with 50 c.c. of water, and a solution

[1] *J. Pharm. Chim.*, 1915, 12, 44.
[2] *Eighth Int. Cong. Appl. Chem.*, 1912, Sect. 1, Orig. Comm. 1, 187.

of twice the theoretical quantity of *p*-nitrophenylhydrazine in 30 c.c. of 30% acetic acid is added. After standing for 5 hours the precipitate is collected on a tared Gooch crucible, washed with 10% acetic acid until the washings no longer give a strong colour with dilute alkali, dried at 105°–110° and weighed. When the benzoic acid present is separately determined by titration with standard alkali solution and deducted from the weight of aldehyde (calculated from that of the hydrazone by multiplying by 0.4400) results showing an accuracy of 99% are obtained.

Hoffmeister has described[1] methods of estimating benzaldehyde in essential oils which depend on the removal of the aldehyde with sodium bisulphite, decomposition of the bisulphite compound with sodium hydroxide solution and subsequent precipitation of the aldehyde with phenylhydrazine.

Assay of Benzaldehyde.—Both Herzog[2] and Heyl[3] adversely criticise the method of the German Pharmacopœia, 5th Edition, for the detection of chlorine compounds in benzaldehyde. Heyl recommends instead that 10–15 drops should be mixed with 1–2 grm. of pure slaked lime, the mixture covered with a thin layer of lime and the whole ignited to redness. The residue is taken up with 5–6 c.c. of water, dissolved in nitric acid and tested with silver nitrate solution. 1 drop of monochlorobenzene, it is stated, can be detected in 50 grm. of benzaldehyde in this way.

Saccharin.

Commercial Saccharin.—The British Pharmacopœia 1914 sets the following standard of purity for saccharin.

"1 grm. heated for 4 hours on a water-bath with 10 c.c. of a mixture of 4 volumes of sulphuric acid and 3 volumes of water completely dissolves, and when the solution is diluted with an equal volume of water and allowed to stand for 24 hours, no crystals separate. Gently warmed for a short time with sulphuric acid no blackening occurs. Ash not more than 0.5%.

Detection of Saccharin in Foods and Beverages.—The methods of detecting saccharin described since the publication of Vol. III, differ chiefly, as did those contained therein, in the process of isolation, the identification still being dependent on its conversion into salicylic acid.

Camilla and Pertusi[4] isolate saccharin from solid foods by digesting on a water-bath with 2% baryta solution and extracting the filtrate, after acidification with sulphuric or phosphoric acid, with a mixture of ether and benzene. A liquid is concentrated on a water-bath, treated with baryta-water, filtered, and the filtrate after acidifying extracted with the ether-benzene mixture.

[1] *Arb. Pharm. Inst. Univ. Berlin*, 1913, 10, 147.
[2] *Ber. Pharm. Ges.*, 1911, 21, 536.
[3] *Apoth. Zeit.*, 1912, 27, 49.
[4] *Giorn. Farm. Chim.*, 1911, 60, 385.

Durand[1] recommends the removal of salicylic acid, if present, by means of bromine. An aqueous extract of the sample to be tested is acidified with phosphoric acid and extracted with ether. The ethereal solution is filtered, evaporated to dryness in a nickel basin and a portion of the residue tested for salicylic acid. If the latter is present, the remainder of the residue is dissolved in hydrochloric acid, an excess of bromine added and the mixture filtered; the salicylic acid is thus completely removed as a bromine derivative. The filtrate is then rendered strongly alkaline with sodium hydroxide, evaporated to dryness, and the residue (or the residue from the ethereal solution if salicylic acid is not present) is fused with soda in the ordinary way for conversion into salicylic acid.

Genth, Jr.[2] extracts saccharin from carbonated beverages with ether, and previous to the extraction of the solution of the alkaline fusion with ether, he exactly neutralises it in the presence of ferric alum solution. The appearance of a violet colour at this stage, renders subsequent operations unnecessary.

Flamand states[3] that the method of extracting saccharin from beer by means of ether or light petroleum, does not give good results when a considerable amount of hops has been used, because the bitter resins extracted simultaneously mask the taste of the saccharin. This difficulty can be met by a preliminary extraction of the beer with benzene, which dissolves the resins, but very little saccharin.

Volhase states[4] that the salicylic acid method is preferable to the recognition of saccharin by conversion of its sulphur into sulphuric acid. Herzfeld and Reischauer,[5] however, use a modification of the latter test to detect saccharin in wine and beer. The residue from the ethereal extract is mixed with magnesium powder and heated cautiously to ignition, the product is dissolved in cold water, filtered, and the filtrate tested with sodium nitroprusside solution, a strong violet colour indicating saccharin.

A new test for saccharin has been described by Wauters[6] and used for the detection of this substance in beer. When saccharin is heated with sulphuric acid and diresorcinol, it yields a product which after treatment with water and filtration gives an intensely violet liquid.

An extraction apparatus for the detection of saccharin in beer has been described by Ledent.[7] It consists of a glass tube 90 cm. long and 1.5 cm. internal diameter fixed vertically and fitted by means of corks, at the top with a stoppered dropping funnel, and at the bottom with a narrow glass tube which is bent round so as to pass up parallel to the wide tube to a height of 35–40 cm. at which point it is bent round and cut off in such a way that the

[1] *J. Ind. Eng. Chem.*, 1913, 5, 987
[2] *Amer. J. Pharm.*, 81, 536.
[3] *Bull. Soc. chim. belg.*, 1913, 26, 477.
[4] *Chem. Zeit.*, 1913, 37, 426.
[5] *Naturwiss. Wochschr.*, 1913, 165.
[6] 7th *Inter. Congr. Appl. Chem.*, London, 1910.
[7] *Analyst*, 1913, 18, 314.

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liquid flowing from it can fall into a flask placed below.   50 c.c. of the beer
are run into the wide tube and then so much ether that the beer begins
to drop from the side tube.   The rest of the beer is then run into the wide
tube drop by drop.   As the drops fall into the ether they are broken up and
the saccharin is extracted, but there is no formation of emulsion, and no
preliminary treatment of the beer such as defecation is necessary.   If neces-
sary the beer can be passed through the apparatus several times.

Commanducci publishes[1] a warning relative to the testing of sweetened
foods and beverages for saccharin.   He states that when solutions of dextrose
and cane-sugar were extracted with a mixture of equal volumes of ether and
light petroleum the residue obtained by evaporation of the ethereal extract
when heated with sulphuric acid and resorcinol and subsequently treated with
alkali and water gave a fluorescent solution the production of which has
always been regarded as characteristic of saccharin.   If, however, the ethereal
extract is washed three or four times with water (which does not remove
saccharin) the residue no longer shows this behaviour.

Camilla and Pertusi[2] have described a method of detecting dulcin,
salicylic acid and saccharin in the presence of one another.

Estimation.—To estimate saccharin in foods rich in fat, starch and
proteins, Tortelli and Piazza[3] recommend the following process.   A
weighed quantity of the sample is mixed with about 18 grm. of fine sand and
10 grm. of slaked lime (liquids are evaporated to the consistence of a paste
after the addition of the sand and lime) and the mixture is extracted four
times with quantities of 50 c.c. of boiling 95% alcohol, 10 c.c. of a saturated
solution of sodium chloride being added each time.   The alcoholic solution
is filtered, the filter washed with a mixture of alcohol and sodium chloride
solution, and the filtrate evaporated to about 80 c.c.   When cold the residual
liquid is mixed with 10 c.c. of saturated sodium chloride solution, and ex-
tracted several times with light petroleum.   After the fatty substances have
been thus removed, the liquid is heated on a water-bath until all traces of
alcohol have been expelled; it is then cooled, acidified with sulphuric acid,
and extracted several times with a mixture of equal volumes of ether and
light petroleum.   The ethereal extracts are washed once with water, filtered
and evaporated and the residue weighed.   If the substance known as "dulcin"
or "sucrol" (*p*-phenetolecarbamide) is present it may be separated by ex-
tracting with ether before acidification.   Dulcin melts at 173°, saccharin
at 223°.   When a trace of the former substance is suspended in water and
heated with mercuric nitrate solution (free from nitric acid) a violet coloura-
tion develops within 15 minutes, and may be made more intense by addition
of a small quantity of lead peroxide.

Possetto and Issoglio[4] separate the saccharin by dialysis.   In the case of

[1] *Boll. chim. farm.*, 1910, 49, 791.
[2] *Giorn. Farm. Chim.*, 1911, 60, 385.
[3] *Zeitsch. Nahr. Genussm.*, 1910, 20, 489.
[4] *Giorn. Farm. Chim.*, 1912, 61, 5.

milk and milk preparations 50 c.c., of jams and fruit jellies 50 grm., and of candied fruits, sweets, or cocoa 30 grm., are made alkaline with sodium carbonate solution and dialysed for 12–24 hours. The yellow solution produced is acidified with phosphoric acid and extracted twice with ether. The ethereal solution is washed, filtered, evaporated and the residue tested for salicylic acid. If this be present it may be separated either by treating the original solution after dialysis with bromine water and filtering, or with permanganate and sulphuric acid and filtering after 12 hours standing, the saccharin being afterwards extracted with ether (after acidifying with phosphoric acid if bromine be employed).

Other methods are described by Condelli[1] and Karas.[2] A method of estimating saccharin and saponin in oil emulsions is published[3] by Carlinfanti and Marzocchi, and a colourimetric method of estimating saccharin in urine has been described by Bloor.[4]

Ceccherelli states[5] that in estimating saccharin treatment of the ether extract with potassium permanganate is not to be recommended, although it is useful in making qualitative tests. Gum tragacanth, extract of gentian and certain other substances after fusion with sodium hydroxide produce colourations with ferric chloride and may therefore interfere with this method of testing for saccharin. Tarugi and Lenci[6] have described the following test, based on the liberation of the amino-group. A minute quantity of saccharin, heated with a few drops of sulphuric acid until white fumes appear, then cooled, diluted with water, neutralised with soda and added to a solution of a crystal of phenol in sodium hydroxide solution, gives a blue colour on addition, drop by drop, of a fresh solution of sodium hypochlorite.

The author concludes that there is at present no satisfactory means of estimating saccharin, applicable to all cases, but the presence of this substance may be regarded as certain if the ether extract, after treatment with permanganate, yields a residue which glistens slightly, contains sulphur, yields an insoluble silver salt containing 37% of silver gives the reactions of salicylic acid after fusion with soda but not before, and also responds to the above test after hydrolysis.

CINNAMIC ACID AND ITS DERIVATIVES.

Cinnamic Acid. β-Phenyl-acrylic Acid.

Detection.—For the detection of cinnamic acid in urine von der Heide and Jakob[7] proceed as follows. A portion of the urine is rendered alkaline, evaporated to a small volume, acidified and extracted with ether. The ethereal solution is shaken with dilute alkali solution. The aqueous alkaline

[1] Stas. sperim. agrar. ital., 1914, 47, 308.
[2] Zeitsch. Nahr. Genussm., 1913, 25, 559.
[3] Boll. chim. farm., 1911, 50, 609.
[4] J. Biol. Chem., 1910, 8, 227.
[5] Ann. Falsif., 1915, 8, 109.
[6] Rend. Soc. Chim. Ital., 1911, 7, 320.
[7] Zeitsch. Unter. Nahr. Genussm., 1910, 19, 137.

solution is heated on a water-bath until all the dissolved ether has been expelled, cooled and treated with 1% potassium permanganate solution; the first few drops of the latter convert any cinnamic acid present into benzaldehyde which may be recognised by its odour. As little as 0.00001 grm. of the acid gives a distinct smell of benzaldehyde.

A similar method is recommended by Schenk and Burmeister.[1] The substance in which cinnamic acid is to be detected is acidified with phosphoric acid and extracted with ether; the ethereal solution is then shaken with sodium carbonate solution, the aqueous layer separated, extracted twice with ether (this is essential, particularly in the case of fruit juices, in order to remove small quantities of furfuraldehyde compounds), and very dilute potassium permanganate solution is added, drop by drop, until the pink colour is only just discharged. The solution is now extracted with ether, the ethereal solution treated with 10 drops of a 5% ethereal solution of phenol and allowed to evaporate at the ordinary temperature. On adding a few drops of concentrated sulphuric acid to the residue a yellow colouration is obtained, even when the quantity of benzaldehyde formed from the cinnamic acid is too small to be identified by its odour. The furfuraldehyde compounds which may be present give, if not removed completely, a faint orange colouration which cannot be mistaken for that yielded by benzaldehyde.

Tunmann has devised[2] a micro-sublimation method of detecting cinnamic acid, especially in resins.

Estimation.—The method previously described by De Jong (Vol. III, p. 438) has been modified by him[3] in order to facilitate the combination of cinnamic acid with bromine. The acid is dissolved in sodium hydroxide solution and reprecipitated with hydrochloric acid. The resulting magma is treated with $N/50$ aqueous solution of bromine until the yellow colouration produced persists for 5 minutes. Then excess of potassium iodide solution is added and the free iodine titrated.

Bongault and Mouchel-la-Fosse[4] for the estimation of cinnamic in the presence of benzoic acid make use of the action between the former acid and sodium hydrogen sulphite. The acid is allowed to combine with a known volume of a standardised solution of the sulphite, the excess of which is then titrated with standard iodine solution.

Cinnamic Aldehyde.

Oil of Cinnamon. Oil of Cassia.—The standard of purity required for cinnamon oil has been considerably modified by the latest edition of the British Pharmacopœia (1914). The latter states that oil of cinnamon is

"Yellow when freshly distilled, gradually becoming reddish. Sp. gr. 1.000 to 1.030; optical rotation —0.5 to —1°; ref. index at 25° 1.565 to 1.580. Soluble in 3 to 4 parts of

[1] *Pharm. Zeit.*, 1915, **60**, 213.
[2] *Pharm. Zentr. h.*, 1913, **54**, 133.
[3] *Rec. trav. chem.*, 1911, **30**, 223.
[4] *Compt. rend.*, 1913, **156**, 396.

alcohol (70%). 1 drop dissolved in 5 c.c. of alcohol (90%) assumes a pale green, but not a blue or brown colouration, on the addition of 1 drop of T. Sol. of ferric chloride [5%] (absence of cinnamon leaf oil and cassia oil). Contains from 55% to 65% of cinnamic aldehyde as determined by the following test:

To 10 c.c. of the oil add 70 c.c. of an aqueous solution (1 in 5) of sodium sulphite and sufficient solution of phenolphthaleïn to give a well-marked pink colouration. Heat the mixture on a water-bath, shake well and neutralise with acetic acid diluted with twice its volume of water; repeat the heating and neutralisation until no further pink colouration is developed, the time occupied being from 30 to 45 minutes. The oily layer which separates on standing, cooled to 15°.5, measures not more than 4.5 or less than 3.5 c.c. (presence of 55% to 65% of cinnamic aldehyde.)"

Cripps and Brown[1] estimate the essential oils in spices (particularly carraway and cinnamon) by heating the latter at 150°–160° (in the case of cinnamon) in a current of air which is afterwards passed through a heated combustion tube, the carbon dioxide formed being absorbed and weighed in the usual way. The weight of carbon found multiplied by 100/80 gives the weight of cinnamon oil.

Adulterations of Oil of Cinnamon.—Hill has pointed out[2] the value of a determination of the refractive index in detecting adulteration of cinnamon-bark oil. Of six samples of the genuine Ceylon oil of the finest quality, the ref. index at 25° varied only between 1.5727 and 1.5767 except for one sample which was more than 9 years old and was very resinous; this gave 1.5797. Two samples of cinnamon-bark oil distilled in England gave the values 1.5614 and 1.5752 respectively. The author gives values for other specimens and for cinnamon-leaf and cassia oils, cinnamic aldehyde, eugenol, pinene and phellandrene, which show that the genuine cinnamon-bark oil is distinguished from cassia oil and cinnamic aldehyde by a low sp. gr. (below 1.04 at 15°), a low ref. index (below 1.58 at 25°), a low aldehyde content (below 65%) and by affording a green colour with ferric chloride solution (cassia oil gives a chocolate brown colour). Adulteration with cinnamon-leaf oil can be recognised by an unduly high proportion of eugenol.

Adulterations of Oil of Cassia.—In the technical valuation of cassia oil, the cinnamic aldehyde is absorbed and the volume of the residue measured. Parry states[3] that most of the cassia oil arriving in London at the present time (1912) is adulterated with resin. Consequently the unabsorbed portion of the oil obtained in the assay has a much higher sp. gr. than cinnamic aldehyde so that an apparent content of 80% (by volume) of cinnamic aldehyde represents a considerably lower actual percentage by weight.

Coumarin.

A method of detecting coumarin in vanilla extracts is described under "Vanillin."

[1] *Analyst*, 1909, **34**, 519; 1910, **35**, 392.
[2] *Chem. and Drug.*, 1910, **76**, 59.
[3] *Perfum. and Essent. Oil Rec.*, 1912, **3**, 46.

Estimation.—Obermayer[1] gives the following method of estimating coumarin in *Melilotus vulgaris* and *officinalis*. 10 grm. of the ground air-dried sample are extracted with ether in an extraction apparatus, and the ether is then evaporated in a 500 c.c. flask. The residue is treated with 300 c.c. of calcium chloride solution (1000 grm. in 3 litres) and distilled until the mass shows signs of solidifying. The heat should be regulated so that the operation lasts fully an hour. The distillate is diluted to a definite volume and then filtered. An aliquot portion of the filtrate is mixed with zinc sulphate solution, and then with excess of standard potassium permanganate solution and boiled on an asbestos plate for 10 minutes. After cooling, the liquid is filtered through an asbestos filter and the precipitate washed with water. The excess of permanganate in the filtrate and washings is determined in the usual manner by titrating with oxalic acid. The titre of the permanganate solution is best verified by a check experiment with pure coumarin.

Aromatic Balsams.

Gum Benzoin.—Reinitzer states[2] that in addition to the benzoates of benzoresinol and siaresinotannol Siam benzoin contains the benzoate of a previously unknown resin-alcohol, *lubanol*. The British Pharmacopœia (1914) gives the following test for Sumatra Benzoin:

"When 0.5 grm. is slowly heated to about 40° with 10 c.c. of solution of potassium permanganate an odour of benzaldehyde is evolved (distinction from Siam benzoin). Not more than 15% insoluble in alcohol (90%). Ash not more than 5%."

Assay.—Cocking and Kettle[3] give the following method to estimate balsamic acids. 5 grm. are extracted with alcohol in a Soxhlet apparatus and the extract hydrolysed with alcoholic potassium hydroxide. The alcohol is evaporated, the residue dissolved in 100 c.c. of water and treated with a slight excess of hydrochloric acid, 5 grm. of light magnesium oxide and 20 c.c. of xylene. The whole is boiled under a reflux condenser for an hour, cooled, the aqueous portion filtered off and the insoluble matter boiled twice with 100 c.c. of water which is allowed to cool before filtering. The combined aqueous extracts are shaken out once with ether, then the balsamic acids are liberated by the addition of hydrochloric acid and removed by ether. The latter is distilled off, the residue dried in a vacuum over sulphuric acid and weighed. To estimate the cinnamic acid, the ethereal residue is left over night in contact with excess of a 5% solution of bromine in carbon tetrachloride and the excess is driven off by evaporation on a water-bath. The residue is evaporated several times with ether dried as before and

[1] *Zeitsch. anal. Chem.*, 1913, 172.
[2] *Arch. Pharm.* 1914, 252, 341.
[3] *Proc. Brit. Pharm. Conf.*, 1914, 13.

weighed. The amount of cinnamic acid is calculated from the increase of weight, one mol. of the acid absorbing two atoms of bromine.

The "free" balsamic acids are estimated by boiling the powdered drug with water, magnesium oxide and xylene and proceeding as above. The acid and ester values are determined on the alcoholic extract of a separate portion of the drug.

CINNAMIC BALSAMS.

Peruvian Balsam.

Analysis.—The British Pharmacopœia 1914 gives the following description and method of assaying Peruvian balsam:

"Insoluble in water; soluble in chloroform. 1 volume is soluble in 1 volume of alcohol (9c %), but on the further addition of 2 or more volumes of alcohol, the whole becomes turbid. Sp. gr. between 1.140 and 1.158. Does not diminish in volume when shaken with an equal bulk of water (absence of ethylic alcohol). When tested by the following method it yields not less than 57% of cinnamein, the saponification value of which is not less than 235.

"Dissolve 1 grm. of the balsam in 30 c.c. of ether and shake in a separating funnel with two successive quantities of 20 c.c. and 10 c.c. of $N/2$ solution of sodium hydroxide. Separate the alkaline solutions, mix and shake with 10 c.c. of ether. Add the second ethereal solution to that previously obtained. Wash the mixed ethereal solutions with two successive quantities of 5 c.c. of water. Transfer the ethereal solution thus washed to a tared wide-mouthed flask, evaporate at a gentle heat until the odour of ether has disappeared, add 1 c.c. of absolute alcohol, dry at 100° for half an hour and weigh. The weight of the cinnamein thus obtained is not less than 0.57 grm. To this residue add 20 c.c. of $N/2$ alcoholic solution of potassium hydroxide, and 20 c.c. of alcohol (90%). Attach a reflux condenser, boil for half an hour, and titrate back with $N/2$ solution of sulphuric acid, solution of phenolphthaleïn being used as indicator. Each gram of the residue thus treated requires not less than 8.4 c.c. of the alkaline solution for complete saponification (corresponding to a saponification value of not less than 235)."

To estimate cinnamein Lehmann and Müller recommend[1] the following method. 5 grm. of water are mixed with 2.5 grm. of the balsam in a 75 c.c. bottle, 30 c.c. of ether are added and the mixture shaken for 1 minute. 5 grm. of sodium hydroxide solution are then added and the mixture again shaken for 1 minute. The bottle is securely corked and set aside bottom upwards for 10 minutes. The cork is then carefully loosened and the aqueous portion allowed to run away till only 3 c.c. are left. 0.5 grm. of gum tragacanth is then added to the contents of the bottle and the whole shaken. After 5 minutes the clear ethereal solution is run into a tared wide-mouthed flask and its weight (w) noted. The solvent is then distilled off, the residue dried at 100° for 30–45 minutes and its weight (w') determined. The percentage of cinnamein (x) in the balsam is given by the following equation: $x = (30w'/w - w')40$. In this method the device of running most of the

[1] Arch. Pharm., 1912, 250, 1.

alkaline solution out of the inverted bottle is criticised as impractical by Frommé[1] who recommends fixing all the solution with gum tragacanth.

For the iodine number of the cinnamein from reliable Peru balsam Jensen found[2] the values 23.8 and 25.5 as against 1.5 for the synthetic ester. Upon fractional distillation of the cinnamein, the first 30% is optically active when derived from the true balsam, but inactive when obtained from the synthetic ester. This author states that benzyl benzoate has sp. gr. 1.121, saponification number 264.1, whilst for benzyl cinnamate the values are 1.098 and 235.3, respectively.

According to Stocker[3] there are undoubtedly pure and genuine balsams on the market which will not dissolve (1 grm.) to a clear solution in a solution of 3.0 grm. of chloral hydrate in 2 grm. of water. He suggests that the test should be modified by using 3.5 grm. of chloral hydrate.

Adulterations of Peruvian Balsam.—K. Dieterich[4] has examined samples of genuine Peru balsam which gave the following values: sp. gr. at 15°, 1.160; acid value 74.02–76.92; saponification value 214.34–243.07; iodine value 22.07 to 25.87; cinnamein 56.56–77.56%; saponification value of the cinnamein 253.61; iodine value of the cinnamein 7.48–7.91; resin esters 24.95%; and insoluble in ether 2.45–4.38%. The results obtained from artificial and "synthetic" balsams, as a whole, differed considerably from these limits, although individual values were frequently normal. The author finds that only the following identification tests are distinctive: Hager's petroleum spirit test; determination of the solubility in alcohol, chloroform, chloral hydrate and carbon bisulphide; the nitric acid test; the zone reactions (Vol. III, p. 458) and the qualitative tests of the Swiss Pharmacopœia. He gives the following new colour test. 1 drop of the sample is shaken with 5 c.c. of light petroleum, and about half the liquid poured off. The remainder is mixed with 5 c.c. of ether and shaken with a few drops (10 to 15) of sulphuric acid, added drop by drop, when genuine Peru balsam gives a violet to blue colouration, whilst artificial products give quite different colours. This reaction is due to the cinnamein of the balsam.

When distilled in a current of steam, Peru balsam yields (Dieterich[5]) about 5% of a thick yellowish oil having an odour like that of the original material. In this way volatile adulterants can be detected. For the oil from the genuine balsam Sortell[6] describes the following characters: sp. gr. 1.0869 at 17°, saponification values 245.7, $[\alpha]_D + 1.876°$. After saponification the oil had sp. gr. 1.002 and $[\alpha]_D + 4°$ at 20°, and it was found to contain benzyl alcohol and peruviol, the greater part of which distilled at 98°/16 mm. Benzoic and cinnamic acids were products of the hydrolysis.

[1] *Caesar and Loretz Jahres-Ber.*, 1912, 20.
[2] *Pharm., J.*, 90, 210.
[3] *Apoth. Zeit.*, 1911, 26, 283.
[4] *Ber. deuts. Pharm. Ges.*, 1913, 23, 622.
[5] *Ber. deuts. Pharm. Ges.*, 1914, 24, 225.
[6] *Ber. deuts. Pharm. Ges.*, 1914, 24, 233.

Dieterich[1] states that irregularities in the preparation of a genuine balsam may cause abnormal colourations in the nitric acid and zone tests.

Tolu Balsam.

The following description of Tolu balsam is given in the British Pharmacopœia, 1914.

"Soluble in alcohol (90%) the solution being acid to litmus. Acid value 107.4 to 147.2; saponification value 170 to 202. If 5 grm. are gently warmed with three successive quantities of 25,15, and 10 c.c. of carbon disulphide, the solution yields when evaporated to dryness a distinctly crystalline residue which when tested as described under 'Styrax Præparatus' yields not less than 1.25 grm. of balsamic acids."

Analysis.—Fleissig states[2] that one of the identity tests of the Swiss Pharmacopœia in which it is directed to treat the substance with alcohol 1:10, should be modified to read specifically 10% ethyl alcohol. Tolu balsam is readily soluble in 95% alcohol. Owing to emulsification there is some difficulty in determining the saponification and acid values. For the latter Merck recommends the following method: 1 grm. of the balsam is dissolved in 50 c.c. of alcohol; 6 c.c. of $N/2$ potassium hydroxide solution are added, then a few drops of phenolphthaleïn solution and, after shaking, 200–300 c.c. of water; the excess of alkali is titrated with $N/2$ hydrochloric acid. The number of c.c. of alkali solution used up by the balsam $\times$ 28 gives the acid value. The saponification value is determined by dissolving 1 grm. of the balsam in 50 c.c. of alcohol, adding 20 c.c. of $N/2$ alcoholic potassium hydroxide and heating for half an hour on a water-bath. Then 200–300 c.c. of water are added and the solution titrated with $N/2$ acid. The number of c.c. of alkali consumed in the saponification $\times$ 28 gives the saponification value.

Liquid Storax.

Analysis.—Umney has proposed[3] the following characters and tests as suitable for determining the purity and quality of the natural drug (as distinguished from that impoverished by extraction of valuable constituents with solvents).

Styrax Purificatus.—The balsam obtained from the trunk of *Liquidambar orientalis*, purified by solution in alcohol and removal of the solvent.

Characters and tests. A brownish-yellow viscous balsam transparent in thin layers, with an agreeable odour and a balsamic taste. Entirely soluble in alcohol and ether. When heated on a water-bath for 1 hour it should not lose more than 5% in weight. When boiled with sulphuric acid and potassium dichromate it evolves an odour of oil of bitter almonds.

[1] *Ber. deuts. Pharm. Ges.*, 1914, 24, 225.
[2] *Schweis. Wochschr.*, 1911, 47, 365.
[3] *Parf. and Ess. Oil. Rec.*, 1911, 2, 126.

The acid and ester values, when determined in the usual manner, should lie between 60 and 90 and 110 and 140, respectively. To estimate the total cinnamic acid, the alcohol is evaporated from the saponified solution and the residue dissolved in 50 c.c. of water. The solution is transferred to a separator, washed with 10 c.c. of ether and the ethereal layer rejected. The aqueous liquid is acidified with N-sulphuric acid and the liberated acids extracted with ether. The ethereal solution is evaporated and the residue extracted with 100 c.c. of boiling water. The extract is filtered whilst hot, the filtrate cooled to 15° and the crystals deposited collected on a tared filter paper. The extraction of the residue is repeated twice with the filtrate heated to boiling and the crystals collected on the same filter. The latter is dried at 100° and weighed. To correct for the solubility of cinnamic acid 0.030 grm. must be added to the weight obtained. The latter should be at least 0.375 grm. from 2.5 grm. of storax.

In criticism of Umney's process, Hill. and Cocking state[1] that cinnamic acid cannot be completely extracted from the mixed organic acids by shaking three or even five times with hot water, and that it cannot be dried to constant weight at 100°. It is preferable to boil the mixed organic acids with water under a reflux condenser, and to dry the acid *in vacuo* over sulphuric acid or to dissolve it in alcohol and titrate with standard alkali.

According to Ahrens,[2] genuine *Styrax liquidus* has the following characters: The substance is mixed with sand and ground with light petroleum. The extract is collected, the solvent distilled off and the residue weighed. The amount soluble in light petroleum varies from 37.6 to 56%, average 45.2%. The acid value of the extract varies from 33.1 to 62.9,. average 46.7, the cold saponification value 191.3 to 201.3, average 196.1. The iodine value of the extract should be determined if the presence of olive oil or castor oil is suspected.

The British Pharmacopœia, 1914, gives the following description of *Styrax Præparatus*.

"Prepared storax is a viscid balsam obtained from the wounded trunk of Liquidambar orientalis, *Mill.*, purified by solution in alcohol, filtration and evaporation of the solvent.

"Entirely soluble in alcohol (90%) and in ether. Boiled with a solution of potassium chromate and sulphuric acid it evolves an odour of benzaldehyde. Loses not more than 5% of its weight when heated in a thin layer on a water-bath for 1 hour. Acid value not less than 60 or more than 90; ester value not less than 100 or more than 146. Yields not less than 20% by weight of cinnamic acid when tested by the following process

"Dissolve 2.5 grm. of the storax in 25 c.c. of $N/2$ alcoholic solution of potassium hydroxide, boil for 1 hour under a reflux condenser, neutralise with $N/2$ solution of sulphuric acid, remove the alcohol by evaporation, and dissolve the residue in 50 c.c. of water. Shake this aqueous solution with 20 c.c. of ether; after separation remove the ethereal layer, wash it with 5 c.c. of water and add the washings to the aqueous solution, rejecting the ethereal liquid. Acidify the aqueous solution with diluted sulphuric acid and shake it with four

[1] *Chem. and Drug.*, 1912, 52.
[2] *Z. öffentl. Chem.*, 1912, 18, 267.

successive portions each of 20 c.c. of ether. Mix the ethereal solutions, wash with a few c.c. of water, transfer to a flask and distil off the ether. To the residue add 100 c.c. of water and boil vigorously for 15 minutes under a reflux condenser. Filter the solution while hot, cool to 15.5°, and collect on a tared filter the crystals of cinnamic acid that have separated. Repeat the extraction of the residue with the filtrate at least three times or until no more cinnamic acid is removed. Press the filter paper and crystals between blotting paper, dry in a desiccator over sulphuric acid and weigh. Add to the weight of the crystals so ascertained 0.03 grm. (representing the average amount of cinnamic acid remaining dissolved in the aqueous liquid). The total weight is not less than 0.5 grm."

SALICYLIC ACID AND ITS ALLIES.

Commercial Salicylic Acid.

The British Pharmacopœia, 1914, gives the following standard of purity for salicylic acid:

"Shaken with a small proportion of water, the mixture filtered and the solution evaporated, there remains a white residue, having no buff-tinted fringe (absence of iron, organic impurities, and colouring matter). When 1 grm. of the acid is dissolved in excess of a cold solution of sodium carbonate, the liquid shaken with an equal volume of ether, and the ethereal solution allowed to evaporate spontaneously, the residue, if any, is free from the odour of phenol (absence of phenol). Arsenic limit, 2 parts per million. No appreciable ash."

Reactions and Detection.—A number of new reactions of salicylic acid have been described. Three of those given by Reichard[1] are as follows: If a little salicylic acid be added to a small quantity of titanic acid, which has been moistened with sulphuric acid and heated for a short time, the mixture set aside for a few hours and then treated with a drop of aqueous potassium hydroxide solution, a fine orange-red colouration is produced. A mixture of salicylic acid and copper sulphate moistened with hydrochloric acid loses its green colour when exposed to the air, but after some days a reddish-violet colouration appears, resembling the biuret indication. When mixed with salicylic acid a concentrated solution of potassium ferricyanide turns dark green. This becomes bluish black on addition of a drop of strong aqueous potassium hydroxide solution, but the colour disappears on shaking, leaving the liquid a slightly green brownish yellow.

Self has described[2] a new colour test for salicylic acid. The substance to be tested is moistened with a cold mixture of equal volumes of concentrated sulphuric acid and 40% formaldehyde and then stirred with a little ammonium vanadate. For 1 mg. of salicylic acid about 2 drops of the liquid and 2–3 mg. of vanadate should be used. In the presence of salicylic acid a Prussian blue colour is produced immediately on adding the vanadate. The test is given by as little as 0.02 mg. of the acid; salicylaldehyde and

[1] *Pharm Zentrh.*, 1910, **51**, 743.
[2] *Pharm J.*, 1915, **94**, 521.

methyl salicylate also respond to it. All other phenolic substances either give no colouration (except that of the reagents alone, an orange colouration changing to green) or give various shades of red, brown or green, usually changing to brown.

According to Wilkie[1] a perceptible precipitate of 2:4:6 tri-iodophenol is produced when equal volumes of $N/10$ iodine solution and $N/10$ sodium carbonate solution, followed after 5 minutes by excess of sulphuric acid, are added to a solution of sodium salicylate containing as little as 1 part of salicylic acid in 870,000.

McCrae states[2] that Kobert's reagent—3 drops of formaldehyde solution in 3 c.c. of sulphuric acid—gives a characteristic rose colouration with salicylic acid.

Barral describes[3] four additional tests for salicylic acid. If 2 drops of a 5% solution of a salicylate are mixed in a test-tube with 2 c.c. of sulphuric acid and 10% sodium nitrite solution added drop by drop with continuous agitation, the liquid becomes in succession orange yellow, reddish orange, blood red with a greenish tinge, gooseberry red. On adding water it changes to orange coloured. When 2–3 c.c. of a 1% salicylic acid solution are warmed with a fragment of ammonium persulphate the size of a pea, the liquid becomes yellow, then brown and finally gives a brownish-black precipitate. On prolonged boiling the liquid becomes colourless. 3 or 4 drops of a dilute salicylic acid solution dissolved in 1–2 c.c. of sulphuric acid give a stable indigo-blue colour with 2 or 3 drops of Mandelin's reagent. With salicylic acid Schlagdenhaufen's reagent in the cold gives a yellow colouration, which deepens to orange and orange brown on warming, with formation of a red precipitate of selenium and evolution of hydrogen selenide. The first, second and fourth of these reactions are given by salicylic esters, the fourth by sulphosalicylic acid and by aspirin.

According to Sherman and Gross[4] Jorissen's test, when carried out in the following manner, is considerably more delicate than that using ferric chloride. The solution to be tested is treated with 4–5 drops of a 10% solution of sodium or potassium nitrite, 4–5 drops of a 50% solution of acetic acid and 1 drop of a 1% solution of copper sulphate, the liquid being shaken after the addition of each reagent. After heating in a boiling water-bath for 45 minutes and cooling, the colour is examined against a white background, a blank test being carried out in a similar manner. In this way 0.000005 to 0.00001 grm. of salicylic acid in aqueous solution can be detected; faint but perceptible indications are obtained with 5–8 c.c. of a 1:1,000,000 solution and with 18–25 c.c. of a 1:3,500,000 solution. Benzoic, cinnamic and tartaric acids, maltol, isomaltol, orcinol, arbutin, resorcinol

[1] J. Soc. Chem. Ind., 1911, 30, 402.
[2] Analyst, 1911, 36, 540.
[3] Bull. Soc. Chim., 1912 [iv], 11, 417.
[4] J. Ind. Eng. Chem., 1911, 3, 492.

and phloridzin do not respond to the Jorissen test. A 1·: 100,000 solution of phenol gives the same colour as a 1 : 1,000,000 solution of salicylic acid. Saligenin gives a red colour at the dilution 1 : 10,000, yellowish tint at 1 : 100,000 but no reaction at 1 : 1,000,000.

During the last four years a number of authors have published methods of detecting salicylic acid in foods and beverages, but for the most part these only differ from those previously known in minor details, e.g., in the variation of the immiscible solvent used.

Thus Stoecklin for the rapid detection in wine and beer recommends[1] the use of dichloroethylene. This author also prefers the Jorissen test in the case of beer and bread.

von der Heide and Jakob[2] extract wine with chloroform. For the detection in milk Philippe[3] (see also Thomann[4]) coagulates with Fehling solution and after acidifying with hydrochloric acid, extracts with ether. Cattini[5] extracts with toluene.

Estimation.—A biochemical method of estimating small quantities of salicyclic acid in the presence of excess of p-hydroxybenzoic acid has been described by Boeseken and Waterman.[6] These authors state that whilst p (and m-) -hydroxybenzoic acid can be used by *Penicillium glaucum* as carbon nutriment, the presence of salicylic acid in quantities of more than 1% causes a retardation in the growth of the organism. By comparing the effect on *Penicillium glaucum* of the mixture under investigation with that of standard mixtures of salicylic acid and p-hydroxybenzoic acid, they claim to be able to determine quantities of salicylic acid varying from 1 to 10% in an excess of p-hydroxybenzoic acid, with an accuracy of about 1%.

Volumetric Methods.—Wilkie states[7] that when action between a phenol (e.g., salicylic acid) and iodine is allowed to proceed for 5 minutes only, the product is wholly tri-iodophenol, whilst this substance after 20 minutes is transformed into tetraiododiphenylenequinone. On this he bases the following method of estimating certain phenols. To the dilute sodium salicylate solution (the acid should be neutralised) equal volumes of $N/10$ iodine and $N/10$ sodium carbonate solution are added. After 5 minutes excess of sulphuric acid is added and the residual iodine titrated with $N/10$ sodium thiosulphate solution. A pronounced fading of the brown colour due to the iodine, or in extreme cases the precipitation of 2 : 4 : 6-tri-iodophenol indicates that too little iodine has been added; in such circumstances more iodine and sodium carbonate solution should be introduced and the estimation completed in the usual manner after 5 minutes. The estimation is best effected in stoppered bottles.

[1] *Ann. Falsif.*, 1912, **5**, 220.
[2] *Zeitsch. Unter. Nahr. Genussm.*, 1910, **19**, 137.
[3] *Mitt. Lebensmittel-unters. Hyg.*, 1911, **2**, 377.
[4] *Schweiz. Wochschr.*, 1912, **50**, 23.
[5] *Boll. chim. farm.*, 1910, **49**, 641.
[6] *Proc. K. Akad. Wettensch. Amsterdam*, 1911, **14**, 604.
[7] *J. Soc. Chem. Ind.*, 1911, **30**, 398.

von Fellenberg ·adversely criticises[1] the process of estimating salicylic acid in jams described by Harry and Mummery (Vol. III, p. 485) on the ground that a portion of the salicylic acid volatilises during the distillation of the ether, that the lead precipitate occludes salicylic acid, that all of the latter is not removed by three extractions with ether, and that the salicylic acid is contaminated with fruit acids which interfere with its colourimetric estimation. The author modifies the process as follows: 15 grm. of the sample are mixed with 50 c.c. of warm water, the mixture neutralised with N-sodium hydroxide solution and treated with ($10-a$) c.c. of sodium citrate solution (prepared by neutralising 35 grm. of the acid with soda and diluting to 500 c.c.), a being the volume of N-sodium hydroxide solution required for the neutralisation. The mixture is now treated successively with 10 c.c. of basic lead acetate solution (sp. gr. 1.24), 10 c.c. of N-sodium hydroxide solution, 5 c.c. of N-hydrochloric acid and 40 c.c. of saturated sodium chloride solution. The addition of these salts prevents salicylic acid from being retained in the lead precipitate formed. The whole mass is now diluted to 150 c.c., filtered and 100 c.c. of the filtrate acidified with 3 c.c. of 20% hydrochloric acid and extracted with five successive quantities of 50 c.c. of ether. The combined ethereal extracts are made alkaline with 10 c.c. of N-sodium hydroxide solution, and the ether removed by distillation. The residual solution is diluted to 50 c.c., acidified with 7 c.c. of 20% hydrochloric acid and treated with a measured excess of $N/50$ bromine solution (prepared by dissolving 0.57 grm. of potassium bromate and 2 grm. of potassium bromide in 1 litre of water). After the lapse of 5 minutes 0.5 c.c. of 10% potassium iodide solution is added for each 10 c.c. of the bromine solution used and the liberated iodine is titrated with $N/50$ thiosulphate solution. Each c.c. of the latter corresponds with 0.00046 grm. of salicylic acid. The bromine and thiosulphate solutions must, in each estimation, be titrated against each other under the same conditions as to dilution, acidity, etc., as in the actual estimation. The difference in the quantities of thiosulphate solution used in the actual estimation and the blank titration gives the amount of salicylic acid present. The author states that the process yields only 90% of the salicyclic acid present, and that jams contain substances which combine additively with bromine, the quantity of such substances (calculated as salicylic acid) being about 0.5 grm. per 1000 grm.

Vierhont has described[2] a method of estimating salicylic acid in fruit juices, in which the substance is acidified with sulphuric acid and extracted with light petroleum, alcohol being added to prevent emulsification. This is stated by Heintz and Limprich[3] to be untrustworthy and these authors publish a very similar method but estimate the acid colourimetrically with ferric chloride.

[1] Zeitsch. Nahr. Genussm., 1910, 20, 63.
[2] Zeitsch. Nahr. Genussm., 1911, 21 664.
[3] Zeitsch. Nahr. Genussm., 1913 23, 706.

In its turn Heintz and Limprich's process has been declared untrustworthy by Serger.

van Raalte[1] for the estimation of salicylic acid in jams, fruit juices, etc., recommends extraction for 5 hours with dichloroethylene.

Gravimetric Method.—Autenrieth and Beuttel state[2] that when phenol, saligenin, salicylic acid or p-hydroxybenzoic acid in aqueous solution is treated at ordinary temperature with excess of bromine a quantitative yield of tribromophenol bromide, $C_6H_2Br_4O$, is obtained, and the action may be used to estimate any one of these substances (in the absence of the others). A weighed quantity of the substance is dissolved in or emulsified with cold water and shaken thoroughly with excess of bromine-water. After standing for 6 hours or more the precipitate is collected on a weighed filter, washed with a small quantity of dilute bromine-water, dried *in vacuo* over sulphuric acid and weighed.

Colourimetric Methods.—According to Linke[3] the colouration given by salicylic acid with ferric chloride is not, as generally assumed, permanent. After 1 hour the violet colouration becomes reddish violet and after 12 hours brownish yellow. But if the solution is stronger than 1 in 50,000 the colour does not change for several days. Linke estimates the free salicylic acid in aspirin tablets by grinding one of these with 25 c.c. of water, adding a drop of ferric chloride solution (the *Liquor Ferri sesquichlorati* of the German Pharmacopœia diluted with 25 volumes of water) and comparing the colour produced with that of standard solutions varying in dilution from 1 in 50,000 to 1 in 200,000.

For the estimation of salicylic acid in marmalades Serger recommends[4] the following process: 20 grm. of the substance are diluted with 30 grm. of water and heated almost to boiling. The liquid is filtered, cooled, and 25 c.c. of the filtrate introduced into a 200 c.c. separating funnel. 5 c.c. of dilute sulphuric acid (1:3) and 100 c.c. of a mixture of light petroleum (3 vols.) and chloroform (2 vols.) are added and the mixture shaken for 3–5 minutes. After settling, 50 c.c. of the chloroform-petroleum layer are filtered into a 100 c.c. cylinder, 1 c.c. of 1% ferric chloride solution is added and the liquid diluted with water to 100 c.c. After shaking for 1 minute the mixture is allowed to separate completely (if separation does not occur quickly 5 c.c. of ether are added and the mixture again shaken), the aqueous layer is diluted to 100 c.c. and its colour matched against that of standards prepared by treating 90 c.c. of water with 1 c.c. of 1% ferric chloride solution and measured volumes of 0.1% salicylic acid solution. The colourations compared should not be more intense than that of an $N/500$ potassium permanganate solution.

[1] *Chem. Weekblad*, 1912, **9,** 1004.
[2] *Arch. Pharm.*, 1910, **248,** 112.
[3] *Apoth. Zeit.*, 1911, **26** 1083.
[4] *Zeitsch. Nahr. Genussm.*, 1914, **27,** 319.

Metallic and Alkaloidal Salicylates.

The British Pharmacopœia, 1914, states of *sodium salicylate:*

"2 grm. heated to redness till gases cease to be evolved leave an alkaline residue which, when treated with water, filtered and well washed, yields a clear solution requiring for neutralisation not less than 24.8 c.c. of $N/2$ solution of sulphuric acid. When to a concentrated aqueous solution excess of diluted nitric acid is added, a precipitate is produced which collected, washed and dried responds to the tests described under Acidum Salicylicum, and the filtrate yields not more than the slightest reactions for sulphates or chlorides. Lead limit, 10 parts per million. Arsenic limit, 2 parts per million. 50 to 100 grm. kept in a closed vessel for several days do not evolve the slightest odour of phenol. Dissolves without colouration or effervescence in sulphuric acid (absence of certain organic impurities and of carbonates)."

The following method of estimating salicylates has been described by Seidell.[1] A weighed sample is placed in a 300 c.c. stoppered bottle with 1-2 cc. of carbon tetrachloride and 100 c.c. of water. Bromine vapour is then poured into the mixture until there is a considerable excess after shaking. After half an hour 5 c.c. of carbon disulphide and 5 c.c. of 20% potassium iodide solution are added and the liberated iodine is titrated with $N/10$ thiosulphate solution (after adding a little more iodide no further liberation of iodine should take place). 5 c.c. of 2% potassium iodate solution are then added and the free iodine is again titrated, further additions of potassium iodide and iodate being made to ensure the completion of the action. The iodine estimated by the second titration corresponds with the hydrobromic acid formed by the action of the bromine on the salicylate, two molecules of acid being formed from one molecule of salicylate. Benzoic acid does not react with bromine under these conditions.

Bismuth Salicylate.—This salt is described by the British Pharmacopœia, 1914, as follows:

"Insoluble in water. When shaken with diluted T. Sol. of ferric chloride [5%] a violet colour is produced. Yields not more than the slightest characteristic reaction with the copper test for nitrates. Arsenic limit, 2 parts per million. When 5 grm. are shaken with 50 c.c. of ether, the ethereal solution filtered off and evaporated to dryness leaves not more than 0.005 grm. of residue (limit of free salicylic acid). Yields, when strongly heated, 62-65% of bismuth oxide. Free from silver, lead, calcium, copper, selenium, tellurium and chlorides."

For the assay of this salt Caron and Raquet have described[2] the following process: 0.5 grm. of the sample is boiled for 10 minutes with 50 c.c. of $N/10$ sodium hydroxide and, after diluting to 100 c.c., the liquid is filtered and 3 c.c., 10 c.c., or even 50 c.c. (according to the amount of nitrate supposed to be present) are evaporated to dryness. The residue is well mixed with 1 c.c. of pure sulphuric acid and 10 c.c. of water, and then 10 c.c. of ammonia are added. Owing to the action of the nitric acid on the salicylic acid which is

[1] *Amer. Chem. J.*, 1912, 47, 508.
[2] *Ann. Chim. anal.*, 1911, 16, 177.

also present a nitro-derivative is formed which dissolves in ammonia with a yellow colour. The colour is then matched with that obtained from a mixture containing a known weight of sodium nitrate.

Sodio-theobromine Salicylate.—This compound is described in the British Pharmacopœia, 1914, as follows:

"A white amorphous powder. No odour; taste sweetish and alkaline. Soluble in 1 part of water; soluble in alcohol; insoluble in ether and chloroform. Aqueous solution (1 in 4) alkaline to litmus and colourless; when acidified with acetic acid yields a violet colouration with T. Sol. of ferric chloride [5%], when neutralised with hydrochloric acid gives a white precipitate of theobromine, and the filtrate from this on addition of more of the acid gives a precipitate of salicylic acid; the precipitated theobromine, washed with a little water, yields, when treated with potassium chlorate and hydrochloric acid and the mixture evaporated to dryness in a porcelain dish and the residue exposed to the vapour of solution of ammonia, a purple colour. The aqueous solution also yields precipitates with T. Sol. of mercuric chloride [5%], with solutions of alkaloidal salts and with $N/10$ solution of iodine. Yields not less than 40% of theobromine and 35% of salicylic acid when tested by the following process: Dissolve 2 grm. of the salicylate of theobromine and sodium in 10 c.c. of warm water, slightly acidifying with diluted hydrochloric acid, add solution of ammonia until the reaction is faintly alkaline and set aside for 3 hours at 15.5°, stirring frequently. Collect the precipitated theobromine on a tared filter, wash twice with 10 c.c. of water, dry at 100° and weigh the precipitate; it weighs not less than 0.8 grm. Acidify the filtrate and washings with hydrochloric acid, shake with two successive quantities each of 10 c.c. of ether, evaporate the mixed ethereal solutions, dry the residue at 60° and weigh. It weighs not less than 0.7 grm."

Salicylic Esters.

Methyl Salicylate.—According to the British Pharmacopœia 1914 this ester should have the following properties:

"Slightly soluble in water, readily soluble in alcohol (90%) in glacial acetic acid and in carbon disulphide. Sp. gr. 1.185 to 1.192. B. p. from 219° to 221°. Optically inactive. The solution in alcohol (90%) is neutral or faintly acid to litmus. The aqueous solution is coloured violet by the addition of a drop of T. Sol. of ferric chloride [5%]. Contains not less than 98% of the ester methyl salicylate."

Natural gaultheria or wintergreen oil is given a slightly different description from that of the pure ester in the British Pharmacopœia, 1914:

"Colourless, strong characteristic odour; taste pungent. Sp. gr. 1.180 to 1.187; optical rotation at 25°, 0° to −1°; ref. index 1.537 to 1.539. Soluble in 6 parts of alcohol (70%) at 25°. Contains not less than 99% of esters calculated as methyl salicylate."

Phenyl salicylate, Salol is described as follows in the British Pharmacopœia, 1914:

"Alcoholic solution neutral to litmus; yields a white precipitate with solution of bromine, and a violet colouration with dilute T. Sol. of ferric chloride. When 0.2 grm. is boiled with 5 c.c. of solution of sodium hydroxide (20%) and the cooled solution acidified with hydrochloric acid, the odour of phenol is developed and a crystalline precipitate is formed.

20

Water which has been shaken with Salol is not affected by T. Sol. of ferric chloride (absence of free salicylic acid and of readily soluble salicylates), and yields no characteristic reactions for sulphates or chlorides. No appreciable ash."

Derivatives of Salicylic Acid.

Acetyl-salicylic Acid.—This is described for the first time in the British Pharmacopœia 1914, which sets the following standard:

"When 0.5 grm. is shaken with 20 c.c. of water and 1 drop of T. Sol. of ferric chloride [5%] is added, no violet colouration is produced (absence of salicylic acid). Lead limit, 10 parts per million. Arsenic limit, 2 parts per million. No appreciable ash."

Linke[1] has utilised the ferric chloride reaction for estimating the amount of free salicylic acid in aspirin tablets (see under Salicylic Acid).

Estimation.—For the estimation of acetyl salicylic acid Astruc has described[2] the method originally proposed by the present writer in 1910 (Vol. III, p. 504).

DIHYDROXYBENZOIC ACIDS AND THEIR ALLIES.

Vanillin

Vanilla.—To detect small quantities of coumarin in factitious vanilla extracts Wichmann has described[3] the following method: 25 c.c. of the extract are made slightly acid with sulphuric acid, 25 c.c. of water added and the whole distilled to dryness. 15–25 drops of potassium hydroxide solution (1 : 1) are added to the distillate which is then rapidly evaporated to 5 c.c. and transferred to a test-tube. The water is now boiled off, and the residue fused to a colourless mass. When cold, the residue is dissolved in a few c.c. of water and made slightly acid with 25% sulphuric acid. The solution is distilled, when the salicylic acid formed from the coumarin during the potash fusion, passes over and may be detected by the addition of neutral ferric chloride solution to the distillate.

Dean[4] modifies Wichmann's method as follows: A de-alcoholised sample of the vanilla extract is treated with 5 c.c. of ammonia and extracted with 15 c.c. of ether; coumarin is dissolved by the ether, but vanillin, salicylic acid and saccharin remain in the aqueous layer. The ether extract is evaporated, the residue treated with 5 drops of 50% potassium hydroxide solution, dried, fused, dissolved in a few c.c. of water acidified with sulphuric acid, and extracted with chloroform. The chloroform solution is tested for salicylic acid, formed from the coumarin, by means of ferric chloride.

The author points out that coumarin would interfere with Durand's test

[1] *Apoth. Zeit.*, 1911, 26, 1083.
[2] *J. Pharm. Chim.*, 1913 [vii], 8, 5.
[3] *U. S. Dept. of Agric., Bureau of Chem.*, Circular No. 95, April 6, 1912.
[4] *J. Ind. Eng. Chem.*, 1915, 7, 519.

for saccharin unless it is removed by extraction with an immiscible solvent in the presence of ammonia.

Winton, Albright and Berry publish[1] analyses of 77 samples of vanilla extract prepared from vanilla beans of different varieties, grades, and lengths by the U. S. P. method. They find that the total acidity ranged from 30 to 52 c.c. of $N/10$ alkali per 100 c.c.; acidity other than that due to vanillin (by difference), 14–42 c.c. of $N/10$ alkali; total ash 0.22–0.43 grm. per 100 c.c.; soluble ash 0.18–0.36 grm.; alkalinity of total ash 30–54 c.c. $N/10$ acid; alkalinity of soluble ash 22–40 c.c: $N/10$ acid.

Detection.—Lecomte has proposed a test for vanillin in quinine wines consisting of mixing an ethereal extract of the wine with dilute hydrochloric acid and adding an alcoholic solution of phloroglucinol; a red zone appears at the junction of the two liquids within about 10 minutes if vanillin is present. Chauvin, however, points out[2] that the reaction is also given by furfural and its derivatives, that these are frequently present in the wines used in the preparation of quinine wines, and that the test is not, therefore, characteristic of vanillin.

Estimation.—Hubbard[3] has found the following disadvantages in the official United States method for the colourimetric estimation of vanillin. There is a difficulty in getting the maximum colouration; the original method of adding the ferrous sulphate first gives a deeper colour than the present one where the bromine is added first. It is difficult to add only enough lead cream (lead hydroxide) to decolourise the solution, and when different quantities are added the depth of colour subsequently obtained varies. Dilution of the sample influences the colour. Much larger amounts of ferrous sulphate are necessary in comparison with bromine water to produce a maximum colour. Lead cream forms a yellow crystalline lead vanillin derivative $(C_8H_7O_3)_2Pb$ which renders a quantitative estimation impossible in the presence of lead.

A new colourimetric method of estimating vanillin has been published by Folin and Denis.[4] 5 c.c. of the flavouring extract are mixed with 75 c.c. of water and 4 c.c. of a solution containing 5% each of basic lead and normal lead acetate, and diluted to 100 c.c. with water. The mixture is rapidly filtered, and 5 c.c. of the filtrate are transferred to a 50 c.c. flask, 5 c.c. of a standard solution of vanillin (1 mg. in 10 c.c.) being placed in another similar flask. 5 c.c. of a phosphotungstic-phosphomolybdic acid reagent are added to each flask and after shaking and allowing to stand for 5 minutes the solutions are made up to 50 c.c. with a saturated solution of sodium carbonate and well mixed. After standing for 10 minutes the solutions are filtered and the colours of the filtrates compared in a Duboscq colourimeter. The reagent is prepared by boiling 100 grm. of pure sodium

[1] *J. Ind. Eng. Chem.*, 1915, **7**, 516.
[2] *Ann. Falsif.*, 1914. **7**, 420.
[3] *J. Ind. Eng. Chem.*, 1912, **4**, 669.
[4] *J. Ind. Eng. Chem.*, 1912, **4**, 670.

tungstate and 20 grm. of phosphomolybdic acid (free from nitrates and ammonium salts) with 100 grm. of syrupy (85%) phosphoric acid and 700 c.c. of water for 1½–2 hours, cooling, filtering if necessary and diluting to 1 litre. Coumarin, extract of tonka beans and acetanilide do not give the rich blue colour produced by vanillin, and sugar, caramel, and glycerol do not interfere with the test:

In place of the dilute standard vanillin solution given above, Harder[1] recommends a stronger one—2 grm. in 200 c.c. of 90% alcohol—as being more stable.

Doherty[2] expresses the opinion that the most trustworthy method of estimating vanillin in essence of vanilla is to distil the essence, extract the vanillin from the residue with ether, combine with sodium hydrogen sulphite, filter, decompose with sulphuric acid, extract with chloroform, evaporate, dry *in vacuo* and weigh. He also gives the following quick method: 1 c.c. of the vanilla essence is extracted with ether, the extract evaporated over water and the aqueous solution filtered and diluted to 50 c.c. in a Nessler glass. 10 drops of freshly prepared bromine water, and 10 drops of 10% ferrous sulphate solution are then added and the colour matched against that given by a 0.2% vanillin solution under the same conditions.

In a research on extraction by means of immiscible solvents Marden[3] has found that 99.6% of the vanillin can be removed from a vanilla extract, which has been freed from alcohol, by shaking once with 20 c.c. and 3 times with 15 c.c. of ether (all the coumarin and about 93% of the acetanilide are simultaneously extracted). For the removal of the vanillin from the ethereal solution he states that the original method of Hess and Prescott (shaking 50 c.c. with 10 c.c. of 5% ammonia) is the best, as it removes all the vanillin and only 5% of the acetanilide and 3% of the coumarin.

From a study of methods of estimating aldehydes Feinberg[4] concludes that for vanillin the iodine and bisulphite methods each give results amounting to 95.5% of the theoretical, whilst precipitation with *p*-bromophenylhydrazine and *p*-nitrophenylhydrazine give 99% and 100% (approx.) respectively.

According to Lehmann[5] vanillin from clove oil has a different melting point from that from guaiacol. He states that the adulteration of vanillin can be detected and its amount estimated by observations of the sintering point, the melting point as determined in the German Pharmacopœia (*i.e.*, the temperature at which the substance forms a continuous column of liquid containing solid particles) and the point of clear fusion. The more nearly pure the product, the smaller is the difference in temperature between the first sintering and complete liquefaction.

[1] *J. Ind. Eng. Chem.*, 1913, **5**, 619.
[2] *J. Roy. Soc., New S. Wales*, 1914, **47**, 157.
[3] *J. Ind. Eng. Chem.*, 1914, **6**, 315.
[4] *8th Int. Cong. Appl. Chem.*, 1912, Sect. I, Orig. Comm., 1, 187.
[5] *Chem. Zeit.*, 1914, **38**, 388, 402.

ERRATA IN VOL. III

Page 408, line 8, for "insolubl" read "insoluble."

Page 408, line 2 from bottom, for "oi" read "oil."

Page 494, line 14, for "il" read "oil."

Page 535, line 10 from bottom, for "phloroglucol" read "phloroglucinol."

Page 559, line 3, for "ordinance" read "ordnance."

Page 575, line 11, for "20°" read "−20°." Line 12, for "12°" read "−12°." Line 9 from bottom, for "25 to 30°" read "−25 to −30°."

Page 593, line 9, for "lime" read "brine."

Page 614, line 16 from bottom, for "ordinance" read "ordnance."

Page 615, line 14 from bottom, for "boiling" read "heating."

Page 616, line 17 from bottom, for "ordinance," read "ordnance."

RESINS.

By ERNEST J. PARRY, B. Sc., F. I. C.

Copal.—Kahan[1] gives the following characters for Benin copal:

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M. p.............................................................. 120° to 166°
Acid value.......................................................  101.0
Saponification value.............................................  149.8
Iodine value.....................................................   61.0
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Willner[2] has examined Loango and Sierra Leone copals and finds them to have the following characters:

	Loango	Sierra Leone
Soluble in ether-alcohol............................	98.7%	92.9%
Acid value.......................................	106 to 115	109 to 114
Saponification value.............................	126 to 134	146 to 150

According to Richmond,[3] Manila copal has an acid value of 128 and saponification value 178. He states that it consists essentially of free amorphous acids, a volatile hydrocarbon, an unsaponifiable resin and a body which is probably a lactone. The free acids are stated to have the formula $C_{10}H_{15}O_2$, $C_{22}H_{34}O_4$ and $C_{32}H_{50}O_4$.

A number of copals obtained from British West Africa have been examined recently in the laboratory of the Imperial Institute[4] and the following values recorded.

	Acid value	M. p.
Ashanti...	124	180°
Ashanti...	134	145°
Ashanti...	133	120°
Ashanti...	120	128°
Sekondi...	133	140°-150°
Sekondi...	133	140°-150°
Nigeria........	110	180°

Dammar Resin.—Coffignier[5] gives the following figures for the best known commercial varieties of dammar resin.

[1] Arch. Pharm., 1910, 248, 443.
[2] Arch. Pharm., 1910, 248, 265 and 285.
[3] Philippine J. Sci., 1910, 5, 177.
[4] Bull. Imperial Inst., 1908, 6, 245.
[5] Bull. Soc. Chim., 1911, 9, 549.

Variety	Sp. gr. at 18°	M. p.	Acid value	Sap. value	Insoluble in		
					Alcohol	Ether	Acetone
Padang..........	1.036	95°	31.4	33.7	20.3%	4.5%	14.7%
Borneo..........	1.048	120°	35.1	64.5	23.6	9.6	20.4
Singapore.......	1.057	95°	30.1	39.3	19.1	1.0	14.0
Pontianac.......	1.025	110°	19.9	30.9	22.4	4.1	16.4
Sumatra........	1.004	190°	59.6	64.5	45.5	37.9	45.3
Batjan..........	1.032	105°	18.5	19.6	32.8	3.2	21.3

According to S. Stewart[1] dammar resin may be detected in kauri resin in the following manner. An ethereal or chloroformic solution of kauri resin is not precipitated by alcohol, whereas that of dammar yields a curdy white precipitate. He proposes to extract the sample in a Soxhlet apparatus with absolute alcohol. In the case of kauri resin only vegetable débris and mineral matters are left, but if dammar be present a large amount of chloroform-soluble resin is left undissolved, averaging about 36%. A fair approximation to the amount of dammar present in kauri resin can thus be obtained.

Dragon's Blood.—The commercial variety of this resin (i.e., Sumatra Dragon's blood) is the product of Calamus Draco, a small palm indigenous to Sumatra.

Guaiacum.—E. J. Parry[2] gives the following figures[3] for genuine guaiacum:

	%
Mineral matter	1-4
Acid value	60-70
Soluble in 90% alcohol	87-98
Acid value of acetylated resin	Not above 50
Ester value of acetylated resin	125-150
Methoxyl number	70-85
Soluble in petroleum ether	Not above 2%

If common rosin be used as an adulterant, the amount is approximately indicated by the solubility in petroleum ether.

Starch is sometimes added as an adulterant, so that all suspected samples should be tested with iodine in the usual manner.

Four samples examined by Evans[4] gave acid values 44.8, 56, 53.2 and 45, or for the alcohol-soluble resins 63, 66, 56 and 65. These samples, however, contained from 9.5 to 29.5% of matter insoluble in alcohol, and are therefore in good agreement with Parry's figures.

Squire[5] gives the following figures for guaiacum.

Acid value of crude lump guaiacum	90- 95
Acid value of purified resin	90-100
Acid value of natural tears	70- 75

The pharmacopœia gives 70-80 as the acid value. Squire's figures do not appear to be confirmed by recent observers.

Shellac.—It should be noted that much of the so-called "button lac"

[1] *J. Soc. Chem. Ind.*, 1909, **28**, 348.
[2] *Food and Drugs*, Vol. I, p. 458.
[3] These figures are for genuine guaiacum freed substantially from extraneous matter and practically represent the alcohol-soluble gums.—E. J. P.
[4] *Analytical Notes*, 1912, **7**, 37.
[5] *Companion to the British Pharmacopœia*, 18th ed., p. 582.

manufactured in Germany is largely factitious, consisting to a great extent of various other resins, mixed with a small amount of shellac, in imitation of ordinary native manufactured button lac.

In reference to the test devised by E. J. Parry, described on pages 69–70 of Volume IV., the statement that "as little as 15 % of colophony may be detected by this test" should read "as little as 5%, etc."

The iodine value of shellac is now universally recognised as the best method for the quantitative estimation of colophony in this product. The Hübl method as recommended by E. J. Parry is accepted as the standard method throughout Europe, whilst in America the Wijs' method is preferred. The latter method generally indicates a higher percentage of rosin than is shown by the Hübl method.   In reference to the method of shellac analysis described by Endemann[1] which does not appear to be of much practical value, the author has published some further details, which do not in any way alter the value of the process.[2]

Vaubel[3] prefers the bromine absorption to the iodine value, as a criterion of the amount of rosin present.   He determines this value as follows: 5 grm. of finely powdered shellac are shaken in a closed flask with 100 c.c. of chloroform or carbon tetrachloride for about 30 minutes, after which 100 c.c. of water, 20 c.c. of strong hydrochloric acid, and 10 grm. of potassium bromide are added.   A solution of potassium bromate (2%) is then added, 1 c.c. at a time, until the liquid assumes a yellow tint which persists for 30 minutes after the flask has been well shaken.   Vaubel states that, determined in this manner, the bromine value of pure shellac is almost 8, whilst that of rosin is 120 to 130.

A useful summary which, however, brings out nothing new, on the analysis of shellac appears in the *J. Soc. Chem. Ind.*, 1911, **30**, 780, by Langmuir and White.

Canada Balsam.—Canada Balsam contains if pure 70 to 85% of resin, the remainder being essential oil.   The resin contains about 20% of an indifferent resene, 20% of amorphous canadinic acid, and 60% of a mixture of α- and β-canadolinic acids, with a trace of crystalline canadolic acid.

The Balsam has the following characters:

Sp. gr.	0.983 to 0.997
Optical rotation	+1° to +5°
Ref. index	1.5175 to 1.5220
Acid value	80 to 90
Ester value	4 to 8

The essential oil has the following characters:

B. p.	160° to 168°
Sp. gr.	0.862 to 0.865
Optical rotation	−26° to −36°
Ref. index	1.4720 to 1.4770
Esters (as bornyl acetate)	Under 1%

The principal constituent of the essential oil is *l*-pinene.

[1] Vol. IV, p. 72.
[2] *Zeit. angew. Chem.*, 1909, **22**, 676.
[3] *Chem. Zeit.*, 1910, **34**, 991.

Copaiba.

Detection of African Copaiba.—Cocking[1] has suggested a method of detecting the so-called African copaiba in genuine copaiba balsam. It consists in distilling the balsam *in vacuo* or with steam and afterwards dividing the resulting oil into 10 equal fractions by distillation *in vacuo*, determining the rotation of these fractions. With pure Maranham and Pará balsam oils all the fractions were lævorotatory and showed a very gradual increase in rotation from the first to the tenth fractions. The differences between the rotations of the tenth and the first fractions ("difference value") were invariably negative and ranged in the case of the separate oil from -3.7 to $-7.6°$. The rotation of the first fraction was a little below that of the original oil. African copaiba balsam yields an oil of which the separate fractions were dextrorotatory. In this case the rotations show a decidedly higher rate of increase than in the American balsam oil, for which reason the difference value is also much greater, whilst moreover it is positive (about $+23°$). Gurjun balsam oil gave exclusively lævorotatory fractions. The rotation of the first fraction was higher than that of the original oil, after this it decreased with each fraction, so that here also the difference value is positive ($+44.4°$). Between the ninth and the tenth fraction there was a sudden decline. In the writer's opinion this method is useless and the deductions drawn quite unwarranted. Further, the slightest deviation from a given method of distillation will give appreciably different results.

Evans[2] gives the following figures for 18 pure and 11 adulterated samples of copaiba. In all cases the essential oil was distilled *in vacuo*.

PURE COPAIBAS.

	Balsam values				Essential oil values		
	Acid value	Acid value, non-oily portion	$n_D 15°$	Ess. oil, per cent.	Sp. gr.	$n_D 15°$	a_D
Para							
1	31.1	107.2	1.5073	71.0	0.904	1.4994	$-30° \ 30'$
2	30.8	102.6	1.5085	70.0	0.903	1.4993	$-31° \ 30'$
3	66.5	162.0	1.5094	59.0	0.906	1.4983	$-17° \ 40'$
4	67.7	118.0	1.5107	42.5	0.895	1.4943	$-29° \ 0'$
5	42.6	152.0	1.5076	72.0	0.903	1.4999	$-14° \ 28'$
6	34.0	151.0	1.5107	77.5	0.9065	1.5026	$-18° \ 45'$
Maracaibo							
7	91.4	170.0	1.5172	46.0	0.902	1.4986	$- \ 8° \ 20'$
8	80.0	143.0	1.5151	44.0	0.897	1.4971	$-10° \ 0'$
9	80.2	143.0	1.5136	44.0	0.899	1.4975	$- \ 9° \ 55'$
10	79.1	143.0	1.5183	44.6	0.903	1.4988	$- \ 9° \ 5'$
Maranham							
11	81.3	138.0	1.5137	41.0	0.8985	1.4968	$-17° \ 52'$
12	86.8	150.0	1.516	42.0	0.9045	1.4992	$-19° \ 12'$
13	78.2	147.5	1.5135	47.0	0.901	1.497	$-17° \ 40'$
14	85.4	158.1	1.5152	46.0	0.8985	1.4966	$-21° \ 41'$
15	84.0	168.0	1.515	50.0	0.903	1.4978	$-16°$
16	89.6	160.0	1.5133	44.0	0.902	1.4977	$-19°$
17	86.8	144.5	1.5137	40.0	0.900	1.4967	$-19°$
18	72.4	120.8	1.5098	44.0	0.8965	1.494	$-20° \ 30'$

[1] *Chem. and Druggist*, 1910, **77**, 119.
[2] *Analytical Notes*, 1914, **8**, 28.

ADULTERATED COPAIBAS.

	Balsam values				Essential oil values		
	Acid value	Acid value non-oily portion	$n_D 15°$	Ess. oil, per cent.	Sp. gr.	$n_D 15°$	a_D
Para							
19	77.1	167.4	1.5100	54.0	0.8905	1.4929	−29° 12′
20	22.4	187.0	1.500	88.0	0.9065	1.499	− 9° 0′
21	31.8	156.6	1.5057	79.7	0.9045	1.499	− 7° 0′
22	26.0	116.0	1.5048	77.5	0.9025	1.5004	−10° 12′
23	14.5	89.2	1.5023	83.8	0.9085	1.4991	−31° 55′
24	77.3	143.0	1.5117	46.0	0.892	1.4957	−26° 15′
25	80.4	174.0	1.511	54.0	0.8845	1.4934	−34° 15′
Maracaibo							
26	85.2	160.7	1.5128	47.0	0.910	1.4975	− 0° 10′
27	87.5	168.3	1.5177	48.0	0.9035	1.4981	− 5° 32′
28	78.0	147.0	1.512	47.0	0.903	1.4981	− 7° 45′
29	82.2	153.9	1.5176	46.6	0.901	1.4987	−10° 20′

Deussen and Eger[1] have devised a new method for the examination of oil of copaiba, in order to decide whether the balsam is pure or not. The oil specially considered is that of African copaiba. The method is based on the fact that African copaiba oil contains much more cadinène than does Para copaiba oil. The dihydrochloride of cadinene melts at 117°–118°, and that of caryophyllene, the principal sesquiterpene of copaiba oil, at 69°–70°. By fractionating the oil, as indicated below, and preparing the dihydrochloride from the suitable fractions, a comparison of the melting points of the crude products is possible, and this will give marked indications as to the presence or absence of African copaiba. The dihydrochloride is prepared by dissolving the fraction in ether, and saturating with dry hydrogen chloride gas, washing the separated crystals with ether, and drying on a porous plate. The melting points of the dihydrochlorides obtained from pure and from adulterated oil are shown in the appended table:

	Fractions at 9 mm.			
	114°–117°	117°–122°	122°–129°	129°–132°
(1) Pure Para Oil—				
Rotation	−8.80°	−11.37°	−12.65°	....
Yield	....	....	23%	15%
M. p.	....	....	75°	76°
(2) +5% African Oil—				
Rotation	−8.88°	−10.25°	−11.33°	....
Yield	....	....	25%	{ very small amount
M. p.	....	....	85°	110°
(3) +10% African Oil—				
Rotation	−6.70°	− 7.85°	− 7.25′	− 4°
Yield	....	....	18.5%	10%
M. p.	....	....	110–112°	115°
(4) +20% African Oil—				
Rotation	−6.20°	− 6.45°	− 1.94°	+3.66°
Yield	....	....	29%	19%
M. p.	....	....	114–115°	116–117°

The authors have also shown that caryophyllene yields, when treated with NO_2 in ethereal solution, a crystalline nitro-compound, which they have somewhat unhappily termed nitro-caryophyllene, of the formula

[1] *Chem. Zeit.*, 1912, 561.

$C_{12}H_{19}N_3O_6$; it melts at 159°–160°. The yield of this body obtained from different oils is variable, and will give some indication of the origin of the oil. They give the following results:

Yield obtained

From caryophyllene from clove oil	50–52 %
From Para copaiba oil (1)	9.5–10 %
(2)	15 %
(3)	15 %
(4)	15–16 %
From Maracaibo oil (1)	5–6 %
(2)	3 %
From Maturin oil	8–9 %
From Para oil +10 % gurjun oil	13.3–14.3 %
From Para oil +20 % gurjun oil	11.7–12.7 %
From Para oil +30 % gurjun oil	10.7–11.7 %
From Para oil +50 % gurjun oil	7.7–8.3 %

Gurjun Balsam in Copaiba.—Gurjun balsam may be detected in copaiba by distilling off the essential oil and dissolving 5 or 6 drops in 10 c.c. of glacial acetic acid mixed with 5 drops of nitric acid. A marked violet-pink colour is developed, its intensity depending on the amount of gurjun oil present. If no colouration results after 2 minutes, any minute colouration occurring afterwards may be neglected, as pure copaiba will occasionally give such a faint indication.

Characters of Copaiba Oil.—The following figures are given by Gildemeister for various types of oil of copaiba (*Die Ätherischen Oele*, 2nd Edition, Vol. II, p. 616).

Balsam	Sp. gr.	Rotn.	Ref. index	Acid value	Ester value
Para	0.886–0.910	−7° to −33°	1.493–1.502	0–1.9	0– 4
Maracaibo	0.900–0.905	−2° 30′ to −12°	1.498	0.9–1.0	1– 1.6
Bahia	0.888–0.909	−8° to −28°	1.494–1.497	0.5–7.9	0–14.9
Maranham	0.896–0.905	−1° 30′ to −22°			
Cartagena	0.894–0.910	−2° 30′ to −23°			
Maturin	0.899–0.904	−7° 30′ to −10° 10′	1.497–1.500	0–0.6	0.9– 3.6
Angostura	0.916	−2° 20′	1.502	10.9	0
Guiana (British)	0.924	−9°			
Guiana (Dutch)	0.903–0.906	−7° 30′ to −10° 30′		0	6.7
Bolivia	0.916	+18°	1.505	1.1	0.5

Composition of African Copaiba Oil.—H. von Soden[1] has examined this oil, the sample in question having a sp. gr. 0.9215, optical rotation +21°, and b. p. from 266° to 270°. The ester value is about 5–6, and the ester value after acetylation about 10. The principal constituent found was cadinene. Schimmel and Co., however,[2] have shown that this sesquiterpene, although very closely related to cadinene, and yielding cadinene hydrochloride, is not, in fact, identical with cadinene. Traces of β-caryophyllene are also present.

Ammoniacum.

●

Genuine samples of ammoniacum, examined by Squire[3] had the following characters:

[1] *Chem. Zeit.*, 1909, 33, 428.
[2] *Report*, April, 1914, 48.
[3] *Squire's Companion*, 18th Edition, page 130.

	1	*2*	*3*	*4*
Ash	2.15%	2.55%	4.3%	7.05%
Acid value	106.7	101.04	101.04	101.04
Ester value	70.2	64.63	67.44	61.82
Saponfication value	176.9	165.67	168.48	162.86
Gum	23.87%	21.06%	18.26%	9.83%

Harrison and Self,[1] as the result of the examination of 7 samples give 0.08 to 0.20% as the amount of essential oil present in this gum resin, the ref. index of which varies from 1.4747 to 1.4808.

Asafœtida.

Harrison and Self[2] give a number of figures covering their examination of certain samples of asafœtida, and suggest that 1.5% of sulphur in the essential oil, expressed as a percentage of the actual gum resin present might be accepted as a minimum figure. These samples gave the following results:

Nature	Loss on dry-ing, %	Re-sin, %	Ash, %	Oil, %	Oil as per-cent-age of gum resin	Sp. gr. of oil	Ref. index of oil at 20°	Rotation of oil (1 dcm.)	Percent-age of sulphur in oil	Sulphur in oil as percent-age of oil	Sulphur in oil as percent-age of gum resin
Mixed, chiefly soft paste.	22.3	50.1	4.3	17.7	18.5	0.929	1.5014	− 0° 11′	22.3	3.95	4.13
Soft paste	26.0	41.3	3.6	8.3	8.3	0.943	1.5083	−10° 20′	27.3	2.27	2.35
Soft paste	32.2	52.1	3.9	19.6	20.4	0.919	1.4968	+ 4° 13′	17.3	3.37	3.53
Stiff paste	24.4	23.9	43.7	5.5	9.8	0.964	1.5124	− 9° 2′	21.7	1.19	2.13
Soft mass	18.7	20.9	52.6	5.9	12.4	0.973	1.5130	−11° 43′	20.7	1.22	2.57
Soft conglomerate	24.0	56.8	2.7	13.9	14.3	0.973	1.5154	− 9° 29′	23.6	3.28	3.37
Dry conglomerate	18.8	54.1	6.2	11.1	11.8	0.918	1.4951	+10° 58′	17.1	1.90	2.02
Hard mass, or "rock"	7.9	19.4	46.1	4.6	8.5	0.993	1.5259	− 8° 23′	31.4	1.44	2.67
Large tears	18.4	50.5	12.4	6.8	7.8	0.959	1.5089	−10° 23′	21.1	1.43	1.65
Tears				17.1			1.5078		17.5	2.99	
Tears				20.8			1.5037		8.9	1.85	

Sechler and Becker[3] give the following method of detecting ammoniacum and galbanum in asafœtida.

If 2 c.c. of a 1 in 10 aqueous emulsion of asafœtida diluted with 5 c.c. of water is floated on sodium hypobromite solution, an olive-green colour results. Galbanum emulsion gives a similar colour; but ammoniacum gives a cherry-red tint. Ammoniacum mixed with asafœtida gives a transient red colour. The reagent is prepared from sodium hydroxide, 20 grm.; bromine, 5 c.c.; water to 100 c.c. If 2 c.c. of asafœtida emulsion be floated on cold sulphuric acid no perceptible change occurs. Ammoniacum also gives no marked reaction. Galbanum gives a violet colour. On distilling off the essential oil, marked differences in appearance are noted. Oil of asafœtida is colourless and has a refractive index = 1.4974. The

[1] *Year Book of Pharmacy*, 1912, 431.
[2] *Year Book of Pharmacy*, 1912, 420.
[3] *Amer. Jour. Pharm.*, 1912, 84, 4.

gum resin yields about 4% of oil. Ammoniacum only yields 0.2% of dark yellow volatile oil which distils with difficulty; its ref. index is 1.4765. Galbanum yields 20% of light yellow oil with the ref. index 1.4840. Consequently the presence of colour in the oil distilled from asafœtida should be regarded as suspicious. The ref. index should not fall below 1.496. The ref. index of a mixture of asafœtida and ammoniacum was 1.4959, that from asafœtida and galbanum 1.4929.

In most cases, however, the ref. index of oil of asafœtida exceeds 1.5000.

The United States Customs Authorities recently laid down a limit for the "lead number" of genuine asafœtida. The method of determining this figure is as follows:

Sufficient of the sample (about 20 grm.) is taken to furnish between 5 and 10 grm. of the resin. The alcohol-insoluble material is determined in the usual manner. The first two filtrates, representing the major part of the sample, are transferred to a casserole, and the alcohol evaporated on the steam-bath. The resin is then dissolved in ether, filtered, transferred to a separator, and washed with water until the aqueous layer separates clear without any milkiness. The ether solution is filtered through a dry paper into a flask or beaker, and the solvent evaporated on the steam-bath.

Into a small tared beaker weigh roughly about 1.1 to 1.2 grm. of the resin prepared as above, and dry in the air-bath at 110° C. for 5 hours. Place in a desiccator, cool, and weigh. Dissolve in 20 c.c. of 95% alcohol, boiling gently until the resin is in solution. Transfer to a graduated 100 c.c. flask, washing the beaker with hot 95% alcohol, care being taken that the final volume does not exceed 70 c.c. Add 25 c.c. of the alcoholic lead acetate solution (described below), and allow to stand over night. Make up to the mark, filter through a fluted paper, and pipette into a beaker an aliquot of 25 c.c., add 10 c.c. of water, and evaporate to 10 c.c.; add 5 c.c. of 10% sulphuric acid , and then 100 c.c. of alcohol, stirring vigorously to dissolve any separated resin. Filter off the lead sulphate on a tared Gooch crucible, and determine the lead in the usual way.

Carry out a blank on the alcoholic lead acetate solution, and calculate the amount of lead absorbed by 1 grm. of the dried resin. The number of milligrams of lead per gram is the lead number.

A good asafœtida should, according to the above authorities, have a lead number of at least 200.

The alcoholic lead acetate solution is thus prepared:

Dissolve 5 grm. of lead acetate in 20 c.c. of water, and add 80 c.c. of 95% alcohol. A turbidity generally results due to the precipitation of lead carbonate caused by the carbon dioxide contained in the alcohol. Allow to stand over night. The clear supernatant liquid can then be used without filtration for the determination of the lead number.

It will be seen that the "lead number" is thus a measure of the con-

stituent (or constituents) of the ether-soluble portion of the resin which form a lead compound insoluble in alcohol.

The writer,[1] however, showed that this test is misleading and valueless. Rippetoe[2] and Harrison and Self[3] have confirmed the writer's conclusions that this test is worthless.

Elemi.

Manila elemi contains up to 30% of an essential oil containing much terpenes and having a sp. gr. varying between 0.875 and 0.910, and is dextrorotatory, to the extent of about +30° to +40°. It has a ref. index about 1.4850.

African elemi[4] is derived from other canarium species and three samples examined had the following characters:

	1	2	3
Ash	0.6%	0.53%	0.3%
Acid No.	55.3	37.8	29.4
Sap. No.	71.9	46.2	44.8
Oil, %	8.1	4.4	71.2
Sp. gr. of oil	0.8686	....	0.8451
$[a]_D$ of oil	+50.5°	....	+79.3°

Galbanum.

Good samples of galbanum should not yield more than 10% of ash, which is the limit fixed by the German Pharmacopœia.

The essential oil of galbanum varies considerably according to the age and species of the gum. Samples examined by the writer had sp. gr. between 0.910 and 0.940 and optical rotations from − 5° to +30°. The identified constituents are pinene and cadinene.

Harrison and Self[5] examined two samples which had the following characters:

	Sp. gr.	Ref. index	Opt. rotn.
1	0.908	1.4856	+15° 14′
2	0.955	1.4869	+ 7° 30′

Myrrh.

Myrrh, according to von Friedrichs[6] contains 3 resin acids soluble in ether, α-, β-, and γ-commiphoric acids. The first 2 are isomeric, having the formula $C_{14}H_{18}O_4$, whilst γ-commiphoric acid has the formula $C_{17}H_{22}O_4$.

[1] Chem. and Druggist, 1913, 82, 34.
[2] Amer. Jour. Pharm., 1913, 85, 199.
[3] Pharm. Jour., 1913, [4] 36, 218.
[4] Bull. Imperial Instit., 1908, 6, 252.
[5] Year Book of Pharmacy, 1912, 430.
[6] Arch. Pharm., 1909, 245, 427.

A resin ester of commiphorinic acid $C_{28}H_{36}O_8$ is also present. Two phenolic substances were also isolated, α-*herabomyrrhol* $C_{18}H_{26}O_5$, and β-*herabomyrrhol* $C_{20}H_{26}O_4$. A volatile alcohol, $C_{14}H_{22}O_2$ exists in small quantity, and *heraboresene*, $C_{42}H_{56}O_8$ is present. Two resin acids insoluble in ether have been termed α-*myrrholic acid*, $C_{15}H_{22}O_7$ and β-*myrrholic acid* $C_{25}H_{32}O_4$. The gum present in myrrh is dextrorotatory, $[\alpha]_D = + 23.8°$.

Genuine myrrh should not yield more than 8% of ash, picked samples giving only 3.5 to 6%. Six pure samples examined by the writer had the following characters:

	1	2	3	4	5	6
1. Sol. in 90% alcohol, %	33.8	41.9	38	37.5	36	43
2. Sol. in water, %	29.5	31.2	37	40.5	38.5	34
3. Sol. in petroleum ether, %	19.6	20.1	17.5	18.5	20.8	16.5
Acid value of 1	59	68	66	70	72	66.4
Ester value of 1	108	121	117	131	119	124
Acid value of sample	20.5	27	26	28	23	20.5
Ester value of sample	34	48	45	50	43	50

Oil of Myrrh.—The essential oil of myrrh described in Vol. IV, (p. 103) is not typical and was probably impure. Normal oil of myrrh has a sp. gr. 0.985 to 1.046 and is lævorotatory up to − 90°. Its ref. index is about 1.5360, acid value 5 to 10, and ester value about 45 to 50. Pinene, limonene and dipentene are present, as well as two sesquiterpenes. Eugenol *meta*-cresol and cumic aldehyde are present in traces.

Two pure samples of Somaliland myrrh (*Ogo malmal and Guban malmal*) have been examined in the laboratories of the Imperial Institute.[1] The results obtained are compared in the following table with those from commercial Aden and Somali myrrhs.

	Myrrh I. Ogo malmal	Myrrh II. Guban malmal	Commercial myrrh, Aden type	Commercial myrrh, Somali type
	%	%	%	%
Moisture	10.2	10.5	8.9	12.9
Ash	3.0	5.2	18.0	4.4
Volatile oil	13.8	11.8	(not determined).	
Resin, soluble in alcohol	31.7	29.7	31.0	22.3
Matter insoluble in alcohol	54.5	58.5	60.1	64.8
Consisting of:				
Matter soluble in water (gum)	52.1	56.8	36.2	58.6
Matter insoluble in water (chiefly dirt).	2.4	1.7	23.9	6.2
Acid value	26.5	17.8	19.0	40.5
Saponification value	143.0	130.0	97.0	120.0

ERRATA IN VOL. IV.

Page 6, line 8, delete "abietic" (compare page 23).

Page 69, bottom line, "15%" should read "5%."

Page 96, Manila Elemi apparently is derived from *Canarium Eusonicum* (*Bull. Imp. Inst.*, 1908, **6**, 252).

[1] *Bull. Imp. Instit.*, 1914, **12**, 11.

INDIA-RUBBER, RUBBER SUBSTITUTES AND GUTTA-PERCHA.

By E. W. LEWIS, A. C. G. I.

INDIA-RUBBER.

Although much work has been published on the subject of rubber analysis during the last 3 or 4 years, it cannot be said that any great advance has been made beyond the position recorded in the fourth volume of this work.

India-rubber Latex.—With the analysis of *Funtumia elastica* latex, quoted in Vol. IV, p. 106, may be compared that published by Fickendey,[1] of a sample considered by Schidrowitz[2] to be more normal, and those of two Malay *Hevea* latices,[3] a *Ficus elastica* latex,[4] and a *Castilloa elastica* latex,[4] published by Beadle and Stevens.

	Funtumia	*Hevea*, 4-year old	*Hevea*, 10- year old	*Ficus elastica*	*Castilloa elastica*
	%	%	%	%	%
Water......................	47.68	70.00	60.00	59.5	62.7
India-rubber...............	40.72	27.07	35.62	37.3	31.2
Resins.....................	4.46	1.22	1.65	....	....
Protein....................	....	1.47	2.03	0.4	0.2
Mineral matter.............	0.99[5]	0.24	0.70	0.4	0.9
Lead acetate ppt............	1.98	....	....	....	....
Peptones....................	3.25	....	....	....	....

Chlorogenic and saccharic acids have been found in the latices of *Castilloa elastica* and *Ficus elastica*.[6]

India-rubber.—The presence of *l*-methylinositol in raw Para rubber has been referred to by Pickles and Whitfeild.[7] This substance may appear in the acetone extract of the crude (unwashed) rubber to the extent of 2 to 3%.

Resins.—The optical activity of the resins accompanying india-rubber from various botanical sources[8] has been confirmed as the result of further work by Hinrichsen and his collaborators.[9] All the resins examined, of which

[1] *Tropenpflanzer*, 1909, No. 5. Gummi-Zeit., 1909, 24, 12.
[2] "*Rubber*," London, 1911, page 123.
[3] *Analyst*, 1911, 36, 6.
[4] Rubber Exhibition "Lectures," London, 1908, page 231.
[5] Mg,Ca,Cl,SO₃,P₂O₅,Al,K,Na.
[6] Gorter, *Rec. Trav. Chim.*, 1912, 31, 281.
[7] *Proc. Chem. Soc.*, 1911, 27, 54.
[8] Allen's Comm. Org. Analysis, 1911, Vol. IV, p. 125, footnote 3.
[9] *Zeit. angew. Chem.*, 1911, 24, 725.

the botanical origin was definitely known, were found to be optically active, with the exception of those from *Hevea* rubber; the activity is concentrated mainly in the unsaponifiable constituents. The application of these facts to analytical investigations is, however, attended by considerable practical difficulty, and can hardly be attempted, except by a chemist experienced in rubber work, and under favourable circumstances.

Estimation of India-rubber.—A reliable method, applicable generally, for the direct estimation of the rubber hydrocarbon, whether in the unvulcanised or in the vulcanised state, is still to seek. In the footnote[1] references are given to a number of papers dealing with the tetrabromide method[2] and its application to unvulcanised and vulcanised rubber. Interesting observations upon some of the obstacles to be overcome in the latter case have been made by Caspari.[3] In the case of unvulcanised (raw) rubber, Budde's method[4] as modified by Spence and Galletly[5] may be usefully employed in some instances.

The sample should first be freed from resins by extraction with acetone. 0.15 to 2.0 grm. of the sample is then cut up into small shreds and soaked for 24 hours in 50 c.c. of carbon tetrachloride. 50 c.c. of a solution of 6 c.c. of bromine and 1 grm. of iodine in 1000 c.c. of carbon tetrachloride are added, and the whole is shaken or stirred at intervals during 6 hours, then vigorously shaken up with 50 c.c. of alcohol and allowed to stand overnight. The liquid is decanted through a filter, the precipitated bromide washed, first with a mixture of carbon tetrachloride and alcohol (2 : 1), then with alcohol, and finally covered with carbon disulphide for 3 to 4 hours. 50 c.c. of benzine (petroleum spirit) are now added, and when the suspended matter has settled the liquid is decanted through the original filter; the sediment is washed thoroughly with alcohol, and dried at a low temperature. The dried bromide is intimately mixed with sodium carbonate and potassium nitrate, in a platinum crucible, covered with more of the mixed carbonate and nitrate, and heated carefully, almost to fusion, for half an hour. The cooled mass is dissolved in water, and the bromine estimated either gravimetrically or by adding excess of $N/10$ silver nitrate to the solution previously acidified with nitric acid, and titrating with standard thiocyanate, using iron alum as in-

[1] Becker, *Gummi-Zeit.*, 1911, 25, 531; 1912, 26, 1503.
Boggs, *Report 8th Int. Congress Appl. Chem.*, 1912, Sect. VB, Orig. Comm., 9, 45–48.
Budde, *Gummi-Zeit.*, 1910, 25, 269.
Esch, *Chem. Zeit.*, 1911, 35, 971.
Fendler, *Gummi-Zeit.*, 1910, 25, 311 and 351.
Hinrichsen and others, *Chem. Zeit.*, 1911, 35, 329; 1912, 36, 217.
Hübener, *Chem. Zeit.*, 1910, 34, 1307 and 1315 and 230; 1911, 35, 113. *Gummi-Zeit.*, 1911, 25, 634: 1912, 26, 1711.
Kirchhof, *Gummi-Zeit.*, 1912, 27, 9.
Korneck, *Gummi-Zeit.*, 1910, 25, 4, 42, 77.
Spence, Galletly and Scott, *Gummi-Zeit.*, 1911, 25, 801.
Spence and Galletly, *Gummi Markt*, 1911, 5, 109. *Caoutchouc et Gutta-percha*, 1911, 8, 5313.
Uts, *Gummi-Zeit.*, 1912, 26, 968.
Vaubel, *Gummi-Zeit.*, 1912, 26, 1879.
[2] Vol. IV, p 109–110, 134–136.
[3] *Caoutchouc et Gutta-percha*, 1911, 8, 5289.
[4] *Gummi-Zeit.*, 1909, 24, 4.
[5] *Caoutchouc et Gutta-percha*, 1911, 8, 5313.

dicator. From the amount of combined bromine found the rubber may be calculated by means of the factor 0.425.

Insoluble Impurities.—Such impurities in raw rubber as bark, sand, grit, etc. (but not protein) may be estimated[1] by heating 1 grm. of the sample with 5 to 10 c.c. of nitrobenzene, high boiling-point petroleum spirit, or phenotole—preferably the last—for 30 minutes at 100° C., then slowly raising the temperature to 140° C. and maintaining it at this point for 1 to 1½ hours until the gelatinous mass suddenly liquefies. The solution is then poured into 100 c.c. of benzene, the clear liquid decanted, and the insoluble residue washed with benzene by decantation and finally on a weighed filter.

Nitrogen.—Of the many variations of the Kjeldahl method Schmitz[2] recommends the following for use with raw rubber: 2 to 3 grm. of the sample are dissolved by heating with 45–50 c.c. of concentrated sulphuric acid in a Kjeldahl flask, with funnel in neck. Frothing may be checked by adding a small piece of paraffin. When cool, 0.25 grm. of cupric oxide and 10 grm. of potassium sulphate are added and the liquid is again heated until it becomes quite clear, and of a greenish colour. Then it is more strongly heated for 1 hour. The total time of heating should not however exceed 4–6 hours. Excess of sodium hydroxide is added and the ammonia is distilled off into excess of standard acid, and titrated iodometrically, using potassium iodate. Ammonium sulphate is recommended for standardisation. The author also advises that the particular variation of the Kjeldahl method employed should always be stated with the result.

Pyridine Extraction.—In attempting to estimate the amount of pitchy and bituminous substances present in a sample of vulcanised rubber difficulties are encountered which, in many cases, are still insuperable. The pyridine extract takes no account of any acetone-soluble portions, which constitute a considerable percentage of the various "mineral rubbers" now so largely used, and in their chemical behaviour approximate very closely to other mineral hydrocarbons which may be present in the acetone extract. The difficulty is accentuated by the fact that lightly vulcanised rubber frequently appears in the pyridine extract. In order to minimise the latter difficulty Becker[3] has recommended a carbon disulphide extraction in place of a pyridine extraction. The process is completed much more rapidly and, except in the case of under-cured samples, no appreciable quantity of rubber is dissolved. The fact remains that the carbon disulphide extract constitutes only a portion of the bituminous material present in the original sample, and in this connection the data published by Caspari,[4] showing its distribution as between acetone extract, carbon disulphide extract, and non-extractable organic residue, will be of value, to an experienced rubber analyst, in interpreting the results of an analysis, which can in any case only be approximate.

[1] Beadle and Stevens, *Analyst*, 1912, **37**, 13.
[2] *Gummi-Zeit.*, 1912, **26**, 1877.
[3] *Gummi-Zeit.*, 1911, **25**, 598
[4] *India-rubber Laboratory Practice*, London, 1914, page 5.

Mineral Matter.—The use of anisole or phenetole in dissolving the extracted sample of vulcanised rubber, in order to separate the mineral matter, is recommended by Marcusson and Hinrichsen[1] largely on the ground that the operation can be conducted at so low a temperature that no decomposition occurs of antimony sulphide and other possible ingredients of the mixing. 1 grm. of the acetone-extracted sample is heated with 20 to 30 c.c. of anisole or phenetole in a weighed 100–200 c.c. conical flask at 90–120° C. for 1 to 2 hours. The solution is diluted with benzene, and the mineral matter, separated by means of the centrifugal, is washed in the flask with benzene; after again separating in the centrifugal it is dried at 105° C. and weighed in the flask.

Estimation of Total Sulphur.—The use of nitric acid saturated with bromine as the oxidising agent in Henriques' method[2] is found to be advantageous.[3] As the simplest and most reliable method of estimating sulphur in vulcanised rubber, Spence and Young[4] recommend an electrolytic process based on Gasparini's method.[5] 0.5 grm. (or more, according to the rubber content) of the sample is covered with nitric acid (sp. gr. 1.4) in a beaker (100 mm. × 55 mm.) covered by a clock glass. The action is started by gently warming the beaker on a water-bath, and when the rubber is completely dissolved 30 c.c. of fuming nitric acid (sp. gr. 1.5) are poured into the beaker over the lower face of the clock glass. A current of 3 amp. is passed between platinum electrodes, 1 sq. in. in area, immersed in the liquid, with a potential difference of 6–8 volts between them, for 2 to 3 hours in the case of soft rubber, or for 45 minutes to 1 hour in the case of hard rubbers. The electrodes are then removed and washed with water; 1 grm. of sodium carbonate is added to the solution, which is then evaporated to dryness on the water-bath. The residue is freed from nitrates by evaporating with hydrochloric acid, and the sulphur is estimated as barium sulphate.

[1] *Chem. Zeit.*, 1910, 34, 839 (Cf. this Vol., p. 322).
[2] Vol. IV, p. 139.
[3] Waters and Tuttle, *J. Ind. Eng. Chem.*, 1911, 3, 734.
[4] *J. Ind. Eng. Chem.*, 1912, 4, 413.
[5] *Gazetta.*, 1907, 37 (II), 426.

ESSENTIAL OILS.

By ERNEST J. PARRY, B. Sc., F. I. C.

HYDROCARBONS OF ESSENTIAL OILS.

There exist in essential oils a small number of open chain hydrocarbons of the formulæ $C_{10}H_{16}$ and $C_{15}H_{24}$, which are known as aliphatic or olefinic terpenes and sesquiterpenes. Only a few have been isolated, the principal of which are the following:

	B. p.	Sp. gr.	Ref. index	Source
Myrcene, $C_{10}H_{16}$	167°	0.802	1.4673	Bay oil
Ocimene, $C_{10}H_{16}$	73° (21 mm.)	0.801	1.4681	Basil oil.
A sesquiterpene, $C_{15}H_{24}$	270–280°	0.864	1.5185	Citronella oil.

Terpenes Proper.

Terpinolene has been found as a constituent of the oil of Manila Elemi.

Terpinene.—Three closely related isomeric terpinenes exist, the formula given on page 171 of Vol. IV, representing α-terpinene, which closely resembles the β- and γ-varieties. α-terpinene exists in coriander oil, whilst γ-terpinene is present in ajowan, lemon and coriander oils.

Phellandrene.—The following are the characters of the purest forms of phellandrene hitherto prepared.

	B. p.	Sp. gr.	Rotn.	Ref. index
l-α-phellandrene	174°	0.848	−84°	1.4769
d-α-phellandrene	175°	0.856	+41°	1.4732
β-phellandrene	57° (11 mm.)	0.850	+15°	1.4759

Sylvestrene.—The inactive form of sylvestrene, which has been prepared synthetically by Perkin, is identical with the racemic form of sylvestrene, so that the name *carvestrene* should be replaced by *i*-sylvestrene. It boils at 178°, and yields a dihydrochloride m. p. 52.5° and a dihydrobromide m. p. 48–50°.

Pinene.—Two isomeric pinenes are now recognised, α-pinene and β-pinene (nopinene). Both exist in oil of turpentine, and are very similar in

[1] *Philippine J. Sc.*, 1907. 2, A 17.

properties. The purest forms hitherto produced have the following characters.

	B. p.	Sp. gr.	Rotation	Ref. index	Source
α-pinene	155°	0.863	0°	1.4664	Synthetic.
α-pinene	156°	0.864	±48°	1.4656	Greek turpentine and eucalyptus oil.
β-pinene	165°	0.865	−19° 30′	1.4755	Hyssop oil.
β-pinene	164°	0.8675	−22°	1.4724	Synthetic.

Fenchene.—The characters of this terpene are as follows:[1] sp. gr. 0.866 at 15°, rotation −32°, refractive index 1.4724, and b. p. 156–157°.

Sesquiterpenes

The characters of the principal sesquiterpenes are as follows:

MONOCYCLIC SESQUITERPENES

	B. p.	Sp. gr.	Rotation	Ref. ind.
Bisabolene	262°	0.881	−41°	1.4901
Zingiberene	270°	0.873	−74°	1.4940

BICYCLIC SESQUITERPENES

	B. p.	Sp. gr.	Rotation	Ref. ind.
Cadinene	272°	0.9205	−99°	1.5065
Caryophyllene	259°	0.9085	−8° 40′	1.5002
Selinene	270°	0.923	+35°	1.4986
Santalene	(See under santal wood oil).			

TRICYCLIC SESQUITERPENES

	B. p.	Sp. gr.	Rotation	Ref. ind.
Cedrene	125° (12 mm.)	0.935	−55°	1.5023
Clovene	262°	0.932	....	1.5010
Heerabolene	135° (16 mm.)	0.945	−14°	1.5125

The sesquiterpene humulene has been shown to be an impure form of caryophyllene.

Estimation of Free Acids in Essential Oils.

The estimation of free hydrocyanic acid in essential oils is at times required in the examination of commercial samples. A few years ago, L. W. Andrews[2] described a volumetric method which was based upon the interaction of hydrocyanic acid and mercuric chloride according to the equation $2HCN + HgCl_2 = Hg(CN)_2 + 2HCl$. The free hydrochloric acid generated by the interaction is titrated with $N/10$ potassium hydroxide solution, p-nitrophenol being used as the indicator. The difficulty of this method lies in

[1] *Wallach. Annalen.* 1908, 362, 180.
[2] *Amer. Chem. J.*, 1903, 30, 187.

Andrews' directions that after being diluted with mercuric chloride solution, the liquid has to stand for an hour. Another drawback is the want of clearness in the colour-change of the nitrophenol. According to Rosenthaler[1] the end-point may be more easily observed if iodoeosin is employed as indicator. Rosenthaler also found that the result was equally satisfactory if the action was limited to a short time.

The following are required for the titration:

(1) $N/10$ potassium hydroxide solution and $N/10$ sulphuric acid.

(2) Iodoeosin (in 0.2% alcoholic solution) and ether.

(3) Solution of mercuric chloride.

The estimation is carried out as follows: The liquid under examination, which should contain about 1% of hydrocyanic acid, should be diluted with iodoeosin solution, neutralised with alkali or acid until the solution just shows a pink tinge; mercuric chloride solution is added and the mixture titrated back immediately with alkali, until the colour reappears.

Whether enough mercuric chloride solution has been added is shown by the titrated liquid no longer becoming coloured after the addition of a few drops of this solution. If a colour should appear it is necessary to add more mercuric chloride and to titrate again with potassium hydroxide solution. If there has been overtitration, add an excess of acid and titrate back again. 1 c.c. $N/10$ potash solution = 2.7018 mg. hydrocyanic acid.

The estimation of the total hydrocyanic acid in liquids which contain free hydrocyanic acid and benzaldehydecyanhydrin offers no difficulties. Neutralise with iodoeosin, dilute with excess of $N/10$ potassium hydroxide solution and again shake for 1 minute. Then add acid until decolourisation has been reached and titrate with alkali until the process is completed. In all cases where solutions of unknown strength are concerned, it is advisable, after finishing the titration, once more to add the potassium hydroxide and mercuric chloride solutions, and after allowing the mixture to stand for 5 minutes, to titrate again. The calculation is based upon the following equations:

$$HCN + KOH = KCN + H_2O$$
$$C_6H_5CH(OH)CN + KOH = KCN + C_6H_5CHO + H_2O$$
$$2KCN + HgCl_2 = Hg(CN)_2 + 2KCl$$
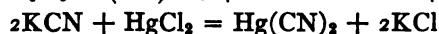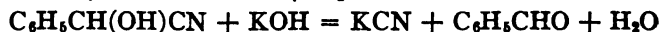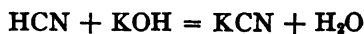
When free hydrocyanic acid has to be estimated in the presence of benzaldehydecyanhydrin it is not sufficient to dilute the neutralised solution with mercuric chloride and to titrate with alkali, because in that case the results are always too high, inasmuch as the nitrile is decomposed by the alkali as the latter is added. In such a case the best mode of procedure is as follows:

The solution under examination is allowed to run into a separating funnel

[1] *Arch. Pharm.*, 1910, 248, 529.

with about 20 c.c. of saturated neutral sodium sulphate solution and neutralised after adding 50 c.c. of ether and 10 drops of iodoeosin solution. Any sodium sulphate which may be precipitated is re-dissolved by adding water. Mercuric chloride solution is next added, the mixture vigorously shaken, and the aqueous liquid separated into a beaker. The solution remaining in the funnel is then extracted once with 20 c.c. of sodium sulphate solution, and again with only a little of the solution. The extracted acid is titrated in the usual way with alkali.

Estimation of Phenols.—One of the most common processes required in the commercial estimation of phenols in essential oils is that of thymol in the oils containing it. According to A. Seidel[1] thymol may be estimated accurately as follows:

Oil containing approximately 0.1 to 0.5 grm. of thymol is placed in a flask of 300 c.c. capacity with 100 c.c. of water. Bromine vapour is then passed into the mixture as long as the brown colour remains permanent after shaking. After standing 20 minutes, 5 c.c. of carbon disulphide and then an excess of a 20% aqueous solution of potassium iodide are added to the liquid. The iodine liberated is titrated with $N/10$ solution of sodium thiosulphate. Afterwards, an excess of a 2% aqueous solution of potassium iodate is added to the liquid and the free iodine again titrated. The thiosulphate solution required in the second titration corresponds with the thymol present. 1 c.c. of $N/10$ thiosulphate solution is equal to 0.007506 grm. of thymol.

Redman, Weith and Brock,[2] have lately found that very accurate results in estimating phenols, including thymol, may be obtained by the method proposed by J. M. Wilkie.[3] The method is based upon the same principle as that of Messinger and Vortmann[4] (see Vol. III, pp. 258 and 301), with the exception that sodium hydrogen carbonate is employed instead of sodium hydroxide. The authors recommend the following process. About 50 c.c. of N-sodium carbonate solution are placed in a glass-stoppered bottle of 500 c.c. capacity and diluted with 100 c.c. of water. 15 c.c. of a solution containing as much of the phenols under examination as corresponds with about an $N/10$ solution is then added. Iodine solution of about $N/30$ strength is then added until a permanent brown colour is obtained. The excess of iodine added should be about 20%. The mixture is then vigourously shaken for 1 minute, diluted with 50 c.c. of $2N$-sulphuric acid, and the excess of iodine titrated with $N/10$ thiosulphate solution, 5 c.c. of a 20% solution of potassium iodide being added and starch used as an indicator. In order that the reaction may be rapid and accurate the mixture should be shaken thoroughly after adding the iodine solution. When this is done, the iodine compound is formed within 1 minute. In the case of thymol the compound formed

[1] *Amer. Chem. J.*, 1912, **47**, 508.
[2] *J. Ind. Eng. Chem.*, 1913, **5**, 831.
[3] *J. Soc. Chem. Ind.*, 1911, **30**, 398.
[4] *Ber.*, 1890, **23**, 2753.

is thymol di-iodide. In order to make sure that any iodine which may have combined with the hydroxyl group is liberated, care should be taken that a little hydriodic acid is always present, hence the addition of the potassium iodide solution before the excess of iodine is titrated back with thiosulphate. Titration is only to be regarded as complete when the blue colouration distinctly returns even after a lapse of 10 minutes.

Estimation of Alcohols in Essential Oils.

In practice several difficulties occur in estimating free alcohols in essential oils. It is, of course, understood that in all cases when the alcohols actually exist as a mixture of two or more bodies, the estimation is in fact only a calculation based on the amount of alkali used in saponification, the alcohols being returned in terms of the predominant constituent.

A difficulty occurs in the case of certain alcohols of which linalol may be taken as a type. This alcohol decomposes to a considerable extent under the action of acetic anhydride, so that the results obtained are considerably below the truth. In order to obtain approximately accurate results, Boulez[1] has recommended the following modification of the ordinary acetylation process. 5 grm. of the oil are diluted with 25 grm. of turpentine which has been carefully rectified over sodium, and then boiled with 40 c.c. of acetic anhydride and 3 to 4 grm. of anhydrous sodium acetate. A blank experiment is necessary in order to allow for the apparent alcohol value of the turpentine. This process give results in the case of linalol which are considerably nearer the truth than with the ordinary acetylation process. Xylene gives even better results than turpentine.

It has been recognised by all workers on the subject that the acetylation process, whilst giving excellent results, varies to the extent of several per cent., according to the exact details of the process used. The following details have been therefore agreed upon by most chemists handling essential oils, so that comparative results may be always obtained.

10 c.c. of the oil are boiled under a reflux condenser with 15 c.c of acetic anhydride and 2 grm. of anhydrous sodium acetate for 2 hours. The mixture is then thoroughly washed with brine until free acid is removed. The washed oil is allowed to stand in contact with anydrous potassium sulphate for 1 hour with occasional shaking. It is then filtered and a weighed quantity, after exact neutralisation with $N/10$ alcoholic potassium hydroxide, saponified for 1 hour under a reflux condenser with an excess of alcoholic potassium hydroxide; the excess of the last-named should be about equal to the amount necessary for saponifying the acetylated oil.

The following estimations are frequently necessary in practice:

(1) The estimation of actual geraniol in citronella oil, that is, the gera-

[1] *Bull. Soc. Chim.* [iv], 1907, 1, 117.

niol as distinguished from the acetylisable constituents, which include the citronellal.

The following process has been communicated by M. V. Boulez to Messrs. Schimmel & Co.[1] He proceeds as follows: "25 or 50 grm. of oil are shaken in an Erlenmeyer flask with 100 or 200 grm. of sodium hydrogen sulphite solution saturated with normal sulphite, and left standing for 2 or 3 hours until the aldehyde has been completely absorbed. 100 or 200 grm. water are then added and the mixture is heated for several hours under a reflux condenser with frequent shaking, until a complete separation has been effected between the oil layer and the aldehyde compound, which has dissolved in the form of a sulphonic acid. The oil is now isolated in a separating funnel, weighed and acetylated. The loss of oil corresponds with the quantity of citronellal which is present in the oil, and the geraniol content is obtained by acetylating the undissolved oil."

Dupont and Labaune have also published a method for the direct estimation of geraniol in citronella oil.[2]

This process depends on the fact that citronellaloxime, produced by shaking with a cold solution of hydroxylamine, is converted, on heating with acetic anhydride, into the nitrile, which is not affected on hydrolysis with alcoholic potassium hydroxide.

The difference in the molecular weight of the nitrile formed and that of citronellal is so small as to be negligible, and the calculation of the percentage of geraniol from the saponification is made by the usual formula.

The method of procedure is as follows: 10 grm. of hydroxylamine hydrochloride are dissolved in 25 c.c. of water; 10 grm. of potassium carbonate separately dissolved in 25 c.c. of water are added, and the mixture filtered. With this solution 10 grm. of the oil are thoroughly shaken for 2 hours at 15°–18° C. The oil is then separated, dried by means of anhydrous sodium sulphate, and acetylated with twice its volume of acetic anhydride in the usual way for 1 hour on a sand-bath under a reflux condenser. The oil is washed, dried and neutralised and a weighed quantity (about 2 grm.) hydrolysed with alcoholic potassium hydroxide.

(2) The separation of citronellol and geraniol in otto of rose has recently become a matter of considerable importance, as the ratio of the amounts of these alcohols present in genuine otto of rose varies within fairly narrow limits. The addition of geraniol modifies this ratio considerably, hence the value of a process for separating the two alcohols. The process is only approximate and therefore must be carried out under definite conditions. It depends upon the fact that formic acid substantially destroys geraniol, whilst it esterifies citronellol. The total alcohols in the sample are determined by the acetylation process above described, and the citronellol determined by

[1] *Report*, October, 1912, page 44.
[2] *Roure-Bertrand's Bulletin*, April, 1913, 13.

formylation in the following manner, the amount of citronellol found being deducted from the total alcohols and the geraniol thus calculated.

10 c.c. of the oil are heated for 1 hour on a water-bath in an acetylation flask with twice its volume of formic acid (sp. gr. 1.226). It has been found advisable to add a few pieces of pumice to regulate the boiling. The contents of the flask are cooled and 100 c.c. of water added, and the whole transferred to a separator. The acid layer is run off, and the oil is washed with water until neutral. It is then dried by means of anhydrous sodium sulphate and filtered. The formylated oil is then neutralised and hydrolysed with aloholic potassium hydroxide in the usual manner, and the percentage of citronellol in the original oil calculated from the following formula:

$$\text{Total citronellol} = \frac{0.156X \times X \cdot 100}{W - (0.028x)}$$

where X is the number of c.c. of normal alcoholic potash absorbed and W the weight of formylated oil.

Umney[1] gives the following typical examples of a number of samples of otto of rose:

	Geraniol by acetylation, %	Citronellol by formylation, %
Bulgarian 1	71.1	30.1
Bugarian 2	70.1	35.7
Bulgarian 3	75.0	36.7
Bulgarian (impure) 4	72.6	13.5
Bulgarian (impure) 5	70.3	17.0
Bulgarian (impure) 6	73.3	22.8
French	65.0	33.1
Anatolian imported direct	66.7	39.2
Anatolian (via Constantinople)	73.6	26.2
Persian (m. p. 29–30°)	38.6	34.5

The last sample, from Persia, is quite abnormal. It has a high melting point and the alcohols contained in it consist very largely of citronellol.

Esters in Essential Oils.

Although the estimation of esters in essential oils is of extreme value, considerable difficulty in correctly interpretating the results obtained has recently been introduced on account of the abuse of scientific knowledge which has led to the preparation of a series of artificial esters, most of which have a saponification value which would indicate the presence of considerably more natural ester than the actual amount of artificial ester used as an adulterant. The principal esters used in connection with these frauds are as follows: terpinyl acetate, glyceryl acetate, ethyl citrate, ethyl oxalate, ethyl succinate, ethyl tartrate, and ethyl phthalate. Apart from the actual identification of the acid constituents of these esters, the following notes in regard to one or two of them will be of value.

[1] *Perfumery Record*, October, 1913, page 329.

Terpinyl Acetate.—This ester is principally found as an adulterant in oil of bergamot, oil of petitgrain, and similar essential oils. The natural ester present in this class of oil consists principally of linalyl acetate. Linalyl acetate is hydrolysed at a considerably more rapid rate than terpinyl acetate. It therefore becomes possible to state with certainty that an artificial ester, probably terpinyl acetate, is present in either of these oils when a marked difference is found between the saponification value as determined at 30 minutes, and that determined in 60 minutes. From the accompanying table[1] it will be seen that hydrolysis of linalyl acetate or of bergamot oil is practically complete in 30 minutes, whereas, the saponification of terpinyl acetate or of bergamot oil adulterated with this ester is much slower.

Ester	Time of saponification, minutes					2 hours
	5	15	30	45	60	
Linalyl acetate (Schimmel & Co.)	191.5	217.5	223.2	223.7	223.1	224.7
Terpinyl acetate	108.2	166.8	209.7	233.4	245.8	262.7
Bergamot oil	80.3	94.5	97.3	97.5	97.8	98.5
Bergamot oil + 5 % terpinyl acetate	82.5	94.8	101.2	102.1	104.7	107.2
Bergamot oil + 10 % terpinyl acetate	79.9	96.4	102.8	105.2	108.3	112.5
Bergamot oil + 25 % terpinyl acetate	78.8	100.6	108.1	116.4	119.0	126.8

Small quantities of terpinyl acetate can be detected by a process of fractional hydrolysis. The following process devised by Messrs. Schimmel & Co., yields most accurate results. Three separate saponifications should be made, using about 2 grm. of oil for each test. 5 c.c. of alcohol are then added, and the free acid neutralised with $N/2$ potassium hydroxide solution. The first saponification should be made for 1 hour with 10 c.c. of $N/2$ potassium hydroxide; the second should be saponified for 2 hours with 20 c.c. $N/2$ alkali, and the third for 1 hour with 10 c.c. of $N/2$ potash and 25 c.c. of 95% alcohol. After saponification the flasks should be cooled quickly by immersion into cold water and titrated at once. In the case of pure oils such as bergamot, petitgrain and lavender, the difference between the saponification for 2 hours and for 1 hour with dilute potassium hydroxide solution will not be more than 3 or 4, whereas, if terpinyl acetate be present, so little as 2% will cause the difference to be from 6 to 7, and 5% will raise it to about 12.

Ethyl Citrate.—Essential oils adulterated with ethyl citrate show a marked turbidity at the commencement of the saponification process. This is due to the separation of potassium citrate which is sparingly soluble in alcohol. After boiling for some time, this turbidity may disappear. Pure bergamot or similar oils do not show this turbidity.

The following method of identifying citric acid is due to Denigès:[2] 10 c.c. of the saponification liquor from which the separated oil has been removed, are shaken with 1 grm. of lead peroxide, and 2 c.c. of solution of mercuric

[1] *Schimmel & Co.'s Report,* Oct. 1910, page 60.
[2] *Bull. Soc. Ph. Bordeaux,* 1898, 33.

sulphate of about 5% strength. After vigorous shaking the liquid is filtered and 5 c.c. of the filtrate heated to boiling point, and a 2% solution of potassium permanganate added drop by drop, constantly stirring until it ceases to become immediately decolourised. If citric acid is present a flocculent pale yellow or white precipitate develops after the first drop.

Glyceryl Acetate.—This adulterant is usually a mixture of glyceryl acetates in which triacetin predominates. Its detection is moderately easy on account of the fact that it is readily soluble in very dilute alcohol, and even fairly soluble in water. Messrs. Schimmel & Co.[1] have proposed the following methods for its detection.

10 c.c. of bergamot oil are mixed in a separating funnel with 10 c.c. of light petroleum and 2.5 c.c. of alcohol, and vigorously shaken up with 20 c.c. of water. The addition of light petroleum and alcohol causes a very rapid separation of the oil and the aqueous liquid, so that the latter can be filtered off when the mixture has been allowed to settle for about 10 minutes. Of the filtrate, 10 c.c. are neutralised with potassium hydroxide and saponified on the water-bath for 1 hour with 5 c.c. $N/2$ potassium hydroxide solution. In the case of pure bergamot oil, the 10 c.c. of filtrate required for saponification.

0.08 c.c. $N/2$ potassium hydroxide solution	= 2.2 mg. KOH
After adding 1 % of glyceryl triacetate. 0.58 c.c.	= 16.2 mg. KOH were used.
After adding 2 % of glyceryl triacetate. 1.43 c.c.	= 40.0 mg. KOH were used.
After adding 5 % of glyceryl triacetate. 2.79 c.c.	= 78.0 mg. KOH were used.

Hence the addition of 1% glyceryl triacetate requires about 15 mg. KOH more for saponification.

Later, Schimmel & Co. proposed omitting the petroleum ether and shaking up 10 c.c. of the oil with 20 c.c. of 5% alcohol. After allowing the 2 layers to separate completely, 10 c.c. of the aqueous layer, which may be filtered, is neutralised, using phenolphthaleïn as indicator, and saponified for 1 hour with 5 c.c. of $N/2$ potassium hydroxide solution. With pure oils not more than 0.1 c.c. of $N/2$ alkali should be required, a higher figure indicating the presence of glyceryl esters. The time taken for the separation of the oil and the aqueous liquids is so long, however, that the use of petroleum ether is preferable. For the positive identification of glyceryl acetate, the same chemists describe the following process.

40 grm. were hydrolysed with the calculated quantity of sodium hydroxide in a concentrated aqueous solution; the solution was then distilled, and, as only water passed over, it was next evaporated to dryness in a dish. The attempt to separate the alcohol (glycerol), which had been split off from the ester, from the sodium salt of the acid by means of an extracting agent failed, because the known solvents took up not only the alcohol but also considerable quantities of the sodium salt. To obviate this difficulty, the sodium salt was decomposed with the calculated quantity of dilute sulphuric acid and the organic acid which was liberated removed by distillation, and subsequently by

[1] *Half-yearly Report*, Oct., 1910, page 61, and April, 1911, page 150.

evaporation. This acid was identified as acetic acid. It was now easy to separate the alcohol of the ester from the residual sodium sulphate by means of ethyl alcohol. When the ethyl alcohol had been evaporated by distilling *in vacuo*, the ester alcohol was identified as glycerol by its b. p. 147° (4 mm.).

As Salamon and Seaber[1] have pointed out, glyceryl acetate is so easily washed out with 5% alcohol, that an adulterated oil, when washed several times with alcohol of this strength, will show a distinctly lower ester value than the original unwashed oil. Pure oils of lavender, bergamot and similar oils show practically no reduction in ester value by such treatment.

Non-volatile Esters.—When non-volatile or practically non-volatile esters such as ethyl citrate are used for the purpose of adulteration, al-
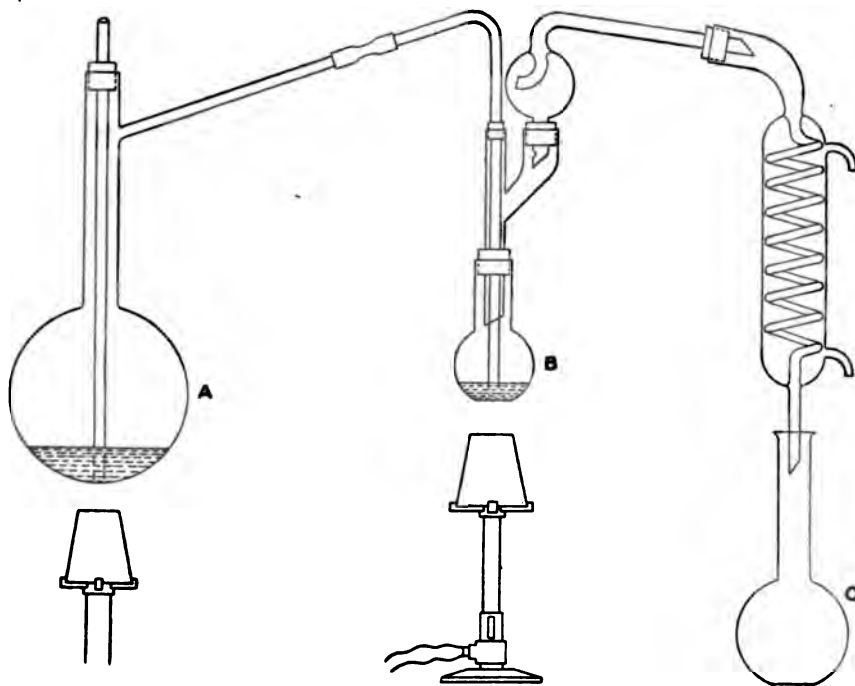
FIG. 13.—Apparatus for estimation of fixed esters.

most the whole of the esters remain in the residue left on evaporating the oil for from 3 to 4 hours on a water-bath. If the weight of this residue is above the normal for a pure oil, non-volatile esters are strongly indicated and may be approximately determined by estimating the saponification value of the residue itself. The process is carried out as follows: 5 grm. of the oil are evaporated in a platinum dish on a water-bath until the weight is practically constant. The residue is washed into a flask and saponified in the

[1] *Perfumery Record*, 1912, 3, 275.

usual manner. Titration should be effected quickly with phenolphthaleïn as indicator. After the neutral point has been reached, the liquid will acquire a red tint in a short time. No notice need be taken of this as it is due to decomposition of bergaptene or similar bodies. The saponification value of the residue from pure bergamot oil varies from about 135 to 180, usually about 170. The addition of 1% of ethyl citrate will raise it about 50, whilst the addition of 2% will raise it by nearly 100. Messrs. Schimmel & Co. have devised the following method of estimating the amount of fixed esters present as an adulterant in this type of oil. The examination of pure bergamot oil has shown that almost the whole of the acid which is combined with the potassium hydroxide on saponification can be distilled off with steam after acidifying the aqueous solution with sulphuric acid. With pure bergamot oil, therefore, only a little more potassium hydroxide solution is used in ascertaining the saponification number than is required for neutralising the acids which have been distilled off. The estimation is carried out with 1.5 to 2.0 grm. of the oil as follows: the acid and ester numbers are ascertained in the usual manner, and the contents of the saponification flask evaporated to dryness after adding a few drops of $N/2$ potassium hydroxide solution. The residue is dissolved in about 5 c.c. of water and acidified with 2 c.c. of dilute sulphuric acid. From the flask A (shown in the accompanying illustration), fitted with an ascending tube, a powerful current of steam is generated, by which, in about half an hour, 250 c.c. is distilled over into the measuring flask C in such a manner that the contents of the saponification flask B are kept down to about 10 c.c. with a small flame. Afterwards 100 c.c. more are carried over in the same manner. The distillate, after a few drops of phenolphthaleïn solution have been added, is titrated with $N/2$ potassium hydroxide solution (or for the sake of accuracy, better still with $N/10$ solution) until it assumes a red colour. The first 250 c.c. contain very nearly the entire volatile acids, as the next 100 c.c. invariably use up only 0.1 to 0.2 c.c. of $N/2$ solution. From the quantity of potash solution required to neutralise the entire distillate the acid number (II) is calculated for the weight of the bergamot oil used. The difference between the saponification number of several pure oils which have been examined and the acid number (II) determined in the manner described above, varied (as shown in the table below, Nos. 1–7) from 5.2 to 6.9.

Hence, unless further examination of pure oils should show a higher figure, oils with a greater difference must be regarded as suspect or adulterated.

In the case of the pure esters (Nos. 12 to 14) here examined the difference between the two values was from 596.4 to 745.8. The addition of such esters to oil of bergamot must, therefore, correspondingly increase the difference of both numbers of the adulterated oil (Nos. 15 to 23 of the table).

	Acid No.	Ester No.	Sap. No.	Acid No. II of the oil	Diff.
		of the oil			
No. 1 Bergamot oil...................................	1.6	88.7	90.3	84.3	6.0
No. 2 Bergamot oil...................................	1.6	90.2	91.8	86.6	5.2
No. 3 Bergamot oil...................................	2.0	101.1	103.1	97.0	6.1
No. 4 Bergamot oil...................................	2.3	112.3	114.6	107.7	6.9
No. 5 Bergamot oil...................................	2.4	100.0	102.4	95.7	6.7
No. 6 Bergamot oil...................................	2.3	96.1	98.4	91.8	6.6
No. 7 Bergamot oil...................................	2.1	98.3	100.4	95.1	5.3
No. 8 Bergamot oil...................................	2.0	98.2	100.2	90.8	9.4
No. 9 Bergamot oil...................................	2.0	103.9	105.9	91.8	14.1
No. 10 Bergamot oil...................................	2.0	102.4	104.4	87.6	16.8
No. 11 Bergamot oil...................................	2.8	103.9	106.7	89.9	16.8
No. 12 Diethyl succinate..............................	....	....	638.4	7.3	631.1
No. 13 Triethyl citrate...............................	0.8	602.6	603.4	7.0	596.4
No. 14 Diethyl oxalate................................	....	....	753.0	7.2	745.8
Bergamot oil after adding:					
No. 15 1% Diethyl succinate..........................	2.1	103.5	105.6	94.1	11.5
No. 16 2% Diethyl succinate..........................	2.1	109.9	112.0	93.1	18.9
No. 17 3% Diethyl succinate..........................	2.1	116.2	118.3	92.8	25.5
No. 18 4% Diethyl succinate..........................	2.1	121.7	123.8	91.8	32.0
No. 19 5% Diethyl succinate..........................	2.1	127.6	129.7	91.5	38.2
No. 20 1% Triethyl citrate...........................	2.1	103.4	105.5	94.1	11.4
No. 21 2% Triethyl citrate...........................	2.1	109.1	111.2	92.8	18.4
No. 22 1% Diethyl oxalate............................	....	....	106.6	94.7	11.9
No. 23 2% Diethyl oxalate............................	....	....	113.3	92.5	20.8
No. 24 2% Glyceryl triacetate........................	2.1	111.2	113.3	108.7	4.6
No. 25 4% Terpinyl acetate...........................	2.4	103.2	105.6	100.0	5.6

Nos. 24 and 25 show that adulteration with the esters of the volatile group cannot be detected by distilling the acids, because in these cases the difference lies of course within the limits for pure oils. The following are average figures for a number of artificial esters.

Ester	Sp. gr. 15° C.	Ref. index 20° C.	Ester, %	Range of b. p.
Ethyl formate, pure.............................	0.908	1.3621	74.9	54°-65°
Ethyl formate, commercial.......................	0.869-0.927	1.360-1.367	59.6- 68.9	55°-80°
Ethyl acetate, pure.............................	0.904	1.3740	100.8	75°- 79°
Ethyl acetate, commercial.......................	0.901-0.904	1.373-1.374	82.8- 94.7	71°- 84°
Ethyl butyrate, pure............................	0.883	1.3922	100.1	110°-120°
Ethyl butyrate, commercial......................	0.883-0.886	1.388-1.492	95.4-100.5	105°-140°
Ethyl succinate.................................	1.042-1.054	1.419-1.421	98.0- 99.5	210°-214°
Ethyl sebacate..................................	0.959-0.966	1.436-1.439	94.9- 96.4	above 300°
Ethyl benzoate.................................	1.048-1.053	1.504-1.545	73.8-100.5	210°-215°
Ethyl cinnamate................................	1.052-1.056	1.557-1.559	99.1-100.6	260°-267°
Ethyl salicylate................................	1.132-1.134	1.521-1.523	96.0- 99.8	225°-230°
Amyl acetate, pure.............................	0.860-0.863	1.397-1.402	89.6- 99.6	130°-140°
Amyl acetate, commercial.......................	0.863-0.870	1.399-1.402	82.2- 88.9	110°-142°
Amyl butyrate, pure............................	0.867	1.4128	99.9	170°-180°
Amyl butyrate, commercial......................	0.857-0.867	1.407-1.412	76.9- 83.4	135°-180°
Amyl valerate, pure.............................	0.858	1.4131	98.7	180°-190°
Amyl valerate, commercial......................	0.850-0.866	1.408-1.413	75.6- 81.8	140°-190°
Amyl salicylate................................	1.048-1.053	1.505-1.508	97.2- 98.7	260°-275°

According to Béhal,[1] the results of the estimation of esters may show differences of between 1 and 3%, especially when formic or acetic esters are in question. He attempts to explain these differences by the suggestion that when esters of high molecular weight are saponified with alcoholic potash, esters of lower molecular weight are first formed, principally ethyl formate and ethyl acetate. Being highly volatile, these are liable to be lost through

[1] *Bull. Soc. Chim.*, 1914, 306.

incomplete condensation; thus benzyl benzoate when heated with alcoholic potash soon develops the odour of ethyl benzoate, and in the same way bismuth salicylate when treated with potash dissolved in methyl alcohol yields methyl salicylate. He assumes that an intermediate di-ester is formed which then splits off the alcohol of high molecular weight. As a result of these observations, Béhal considers that a number of essential oils contain formic esters in quantities hitherto unsuspected. He considers formic acid constitutes an important part of the esters of Algerian oil of geranium.

Estimation of Aldehydes and Ketones.

Hanus[1] proposes the following method for the estimation of cinnamic aldehyde, etc. 10 grm. of finely powdered hydrazine sulphate are dissolved in a solution of 9 grm. of sodium hydroxide in 100 cc. of water, and the alkaline sulphate produced is precipitated by the addition of 100 c.c. of alcohol. After filtering, the solution is warmed, 9 grm. of oxamethane are added in small portions, the whole warmed for half an hour and allowed to cool. The azide separates in crystalline tables, and these are separated and recrystallised. To estimate the aldehyde by means of this reagent, a small quantity, not more than 0.2 grm., of the oil is well shaken in 85 c.c. of water, and about 0.35 grm. of semi-oxamazide in 15 c.c. of hot water is added and the whole well shaken. After 5 or 10 minutes the compound begins to be precipitated, and after standing 24 hours can be collected on a Gooch filter, washed with cold water and dried for a few minutes at 105°. The amount of the precipitate is multiplied by 0.6083 to obtain the amount of aldehyde. The constitution of the semi-oxamazone of cinnamic aldehyde is $NH_2.CO.CO.NH.N : CH.CH :- CH.C_6H_5$.

Feinberg has obtained good results in estimating aldehydes as p-nitrophenylhydrazone by the method of W. Alberda van Ekenstein and J. J. Blanksma.[2] In the case of benzaldehyde he proceeds as follows: 25 c.c. of a 1% benzaldehyde solution (in 12% acetic acid) are diluted with 50 c.c. of water and mixed with 30 c.c. of 30% acetic acid containing double the calculated quantity of p-nitrophenylhydrazine. After 5 hours the precipitate is collected in a Gooch crucible and washed with 10% acetic acid until a well-defined colouration has ceased to ensue upon the addition of alkali. The precipitate is then dried at 105° to 110° and weighed. The benzaldehyde-content is found by multiplying by 0.44. It is necessary to ascertain by titration the quantity of benzoic acid which is present in the benzaldehyde, and to deduct it from the quantity of benzaldehyde used. The average proportion of benzaldehyde found was about 99%. The same method applies to salicylic aldehyde, but in this case filtration may be commenced after 1 hour (factor 0.4747).

[1] Pharm. Central., 1904, 37.
[2] Chem. Zentralbl., 1905, 1, 1277.

For **vanillin** and **anisic aldehyde** Feinberg gives the following method: Dissolve 0.5 grm. of aldehyde in a little alcohol and acetic acid, and after diluting with 75 c.c. of water and warming, mix the solution drop by drop with constant stirring with a solution of p-nitrophenylhydrazine in 2N-hydrochloric acid. Filter after 30 minutes in a Gooch crucible and wash with 2N-hydrochloric acid and afterwards with water until only a faint opalescence is produced by silver nitrate. For the estimation of the anisic aldehyde use the factor 0.50188; for vanillin, 0.5353.

Estimation of Citral.

Various colourimetric processes of estimating citral have recently been devised. Of these, the following give fairly accurate and concordant results.

Hiltner's Method.—R. S. Hiltner[1] bases a method on the fact that solutions of m-phenylenediamine hydrochloride give a yellow colour with solutions of citral, the intensity being dependent on the amount of citral present. This solution, which preferably should be freshly prepared each time, must be quite clear and colourless, and, if necessary, should be made so by treating it with animal charcoal and filtering. The percentage of citral in the extract or oil under examination is determined by comparative tests with a solution of known citral-content (0.25 grm. citral to 250 c.c., dissolved in 50% alcohol).

The test is carried out as follows: 1.5 to 2 grm. lemon oil, or 25 grm. of lemon-extract are diluted to 50 c.c. with 90 to 95% alcohol; 2 c.c. of this solution are poured into the colourimeter-vessel, 10 c.c. m-phenylenediamine solution added, and the whole made up to a fixed volume. Comparative tests are made simultaneously in the same manner with the standard solution referred to above, until the precise shade of colour of the solution under examination is reached. The citral-content of the oil or extract of lemon can be calculated from the quantity of the standard solution taken up in the test. If the citral-content is less than 0.1%, it will be desirable to use a little more of the solution under examination, in order to obtain the intensity of colour which is necessary to give exact results.

Chace's Method.—Chace[2] has devised a method depending on the property of fuchsine-sulphurous acid of giving a red colouration in the presence of aldehydes. The solution of fuchsine-sulphurous acid is prepared as follows:

0.5 grm. of fuchsine is dissolved in 100 c.c. of water and a solution containing 16 grm. of SO_2 is added and when decolourised the whole is made up to 1,000 c.c. The solution must be freshly prepared, that is, not more than 3 or 4 days old. Alcohol of 95% strength is freed from aldehyde by keeping it in contact with caustic alkali for several days and then distilling, and boiling the

[1] *J. Ind. Eng. Chem.*, 1909, 1, 798.
[2] *J. Amer. Chem. Soc.*, 1906, 28, 1472.

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distillate with 25 grm. of *m*-phenylenediamine per litre, for a few hours under a reflux condenser. The alcohol is then finally redistilled.   A standard solution of 0.1% of citral in 50% alcohol is prepared.   A quantity of the oil or essence containing about 0.1 to 0.2 grm. of citral is diluted to 100 c.c. with 50% aldehyde-free alcohol.   4 c.c. of this solution are used for the determination, and are mixed with 20 c.c. of alcohol and 20 c.c. of fuchsine-sulphurous acid solution, and made up to 50 c.c. with alcohol.   After well mixing, the observation tube is kept at about 15° and the colour compared with the necessary standards, from which the amount of citral can be calculated.

Little's Method.—Little[1] prefers the following method depending on the the use of diaminophenol.

The method is easily manipulated and can be conducted at room temperature:

Reagent.—Dissolve 0.2 grm. diaminophenol hydrochloride (commercially known as amidol), in 100 c.c. of 65% by volume alcohol, preferably distilled over potassium hydroxide.   The use of aldehyde-free alcohol does not seem to make any difference in the results, as acetaldehyde has no apparent effect upon the reagent.   The reagent is very readily soluble in 65% alcohol.

Standard Citral Solution.—A solution of pure citral in 50% alcohol, containing 0.001 grm. per c.c.

Solution of Essence or Extract of Lemon.—Weigh from 15 to 25 grm. of the extract for examination, and dilute to 30 or 50 c.c. with 50% alcohol, if a terpeneless extract, making a 50% solution.

Place 2 c.c. of the standard citral solution measured from an accurately graduated pipette, in a 250 mm. colourimeter tube, add 20 c.c. of 65% alcohol, and 15 c.c. of diaminophenol reagent and make up to 50 c.c. with 65% alcohol. Place 2 c.c. of the extract in the other tube with 15 c.c. of the reagent and make up to 50 c.c. with 65% alcohol as before, mixing the contents of both tubes thoroughly and allowing both tubes to remain for 5 to 10 minutes at room temperature, when the maximum colour is reached in both the citral tube and the extract under examination.   The reading and calculation are made at once or a reading can be made at the expiration of 15 or 20 minutes in duplicate.   The calculation of the percentage of citral content in the extract is made by placing the standard citral tube at the 30 mm. mark and adjusting the tube containing the extract under examination so that the 2 small discs of colour as observed through the two immersion tubes are similar in tint.

Kleber[2] uses a method based on the phenylhydrazine reaction, which, as slightly modified by Schimmel & Co., is as follows:

About 2 c.c. of oil are mixed with 10 c.c. of a freshly prepared 2% alcoholic phenylhydrazine solution, and allowed to remain undisturbed during 1 hour

[1] *Amer. Perfumer.* 1914, 74.
[2] *Amer. Perfumer.* 1912, 6, 284.

in a glass-stoppered flask of about 50 c.c. capacity. 20 c.c. of $N/10$ hydrochloric acid are then added and the liquid is mixed by gently moving the flask backwards and forwards. After adding 10 c.c. of benzene the mixture is shaken vigorously and poured into a separating funnel. The acid layer, amounting to about 30 c.c., which separates clearly after the mixture has been left to settle a short time, is then filtered on a small filter.

To 20 c.c. of this filtrate 10 drops of diethyl-orange solution (1 : 2,000) are added and the mixture is titrated with $N/10$ potassium hydroxide solution until a distinct yellow colour appears. From this the quantity in c.c. of $N/10$ potassium hydroxide solution required for 30 c.c. of the filtrate is calculated. For the purpose of estimating the value of the phenylhydrazine solution a blank test without oil is made in a similar manner. If it is found that the quantity of solution used up for 30 c.c. of the filtrate $= a$ in the first, and $= b$ in the second experiment, it follows that the quantity of citral present in the amount of oil under test (s grm.) equals $a - b$ c.c. of $N/10$ solution. Hence, 1 c.c. $N/10$ potassium hydroxide being equal to 0.0152 grm. citral, the percentage proportion of citral in the oil is expressed by the following formula

$$\frac{(a - b) \cdot 1.52}{s}$$

The object of the extraction with benzene is to reclarify the solution, which becomes turbid after the addition of the hydrochloric acid. When this has been done it is easier to recognise the change in the colour during the titration.

Refractive Indices of Essential Oils.

The following table has been brought up to date, the figures having been determined at 20° except where otherwise stated.

Acorus calamus (sweet flag)	1.5028 to 1.5150
Almond (bitter)	1.5320 to 1.5446
Almond (bitter) (S.A.P.)	1.5420 to 1.5460
Angelica	1.4800 to 1.4820
Angostura bark	1.5060 to 1.5085
Aniseed	1.5535 to 1.5565
Bay leaf (Pimenta)	1.4850 to 1.5860 (according to sp. gr.)
Bay leaf (Laurus)	1.4785 to 1.4800
Bergamot	1.4640 to 1.4690
Buchu	1.4740 to 1.4870
Cajuput	1.4600 to 1.4660
Callitris (leaves)	1.4745 to 1.4815
Caraway	1.4870 to 1.4975
Cardamom	1.4610 to 1.4700
Cassia	1.5880 to 1.6045
Cassie (flowers)	1.5120 to 1.5140
Cedar wood	1.4980 to 1.5050
Cedar wood (Libanon)	1.5125 to 1.5134
Chamomile (Anthemis)	1.4450 to 1.4525
Cinnamon bark	1.5900 to 1.5995
Cinnamon leaf	1.5350 to 1.5400
Citronella (Ceylon)	1.4650 to 1.4680
Citronella (Java and Singapore)	1.4800 to 1.4835
Citron	1.4745 to 1.4755
Clove (buds)	1.5300 to 1.5360
Cognac	1.4290 to 1.4300

Copaiba (Angostura)... 1.5010 to 1.5030
Copaiba (Bahia)... 1.4940 to 1.4975
Copaiba (Cartagena).. 1.4950 to 1.5010
Copaiba (Maracaibo).. 1.4975 to 1.5000
Copaiba (Maturin).. 1.4975 to 1.5015
Copaiba (Para)... 1.4930 to 1.5025
Coriander.. 1.4660 to 1.4675
Cubeb... 1.4909 to 1.4965
Cumin... 1.5040 to 1.5060
Cypress (leaves)... 1.4700 to 1.4815

Dill.. 1.4800 to 1.4950

Eucalyptus (B.P. and U.S.P. types)............................. 1.4600 to 1.4675

Fennel.. 1.5250 to 1.5345

Galangal.. 1.4795 to 1.4815
Geranium (Pelargonium).. 1.4620 to 1.4720
Geranium (Cymbopogon).. 1.4720 to 1.4768
Ginger.. 1.4880 to 1.4950
Gingergrass... 1.4780 to 1.4930
Guaiac wood (Bulnesia) at 30°................................. 1.5030 to 1.5050
Gurjun balsam... 1.4940 to 1.5025

Hop... 1.4850 to 1.4920

Juniper (berry)... 1.4769 to 1.4835

Kananga... 1.4810 to 1.5110

Laurel (*Laurus nobilis:* leaves)............................. 1.4650 to 1.4770
Lavender.. 1.4620 to 1.4670
Lemon... 1.4745 to 1.4760
Lemongrass.. 1.4820 to 1.4885
Lime oil (distilled).. 1.4750 to 1.4780
Lime oil expressed)... 1.4800 to 1.4845
Linaloe... 1.4610 to 1.4630

Mace.. 1.4760 to 1.4800
Marjoram.. 1.4750 to 1.4850
Marjoram (Cretic)... 1.5050 to 1.5105
Matico.. 1.4960 to 1.5290
Melissa... 1.4738 to 1.4820
Michelia longifolia (flowers)............................... 1.4500
Mustard... 1.5250 to 1.5281

Neroli.. 1.4748 to 1.4765
Nutmeg.. 1.4760 to 1.4800

Orange.. 1.4725 to 1.4760
Orange (Tangerine).. 1.4760 to 1.4790
Orris (liquid).. 1.4940 to 1.4960

Parsley... 1.4800 to 1.5190
Patchouli... 1.5110 to 1.5150
Pennyroyal.. 1.4800 to 1.4825
Pepper.. 1.4890 to 1.4990
Peppermint.. 1.4640 to 1.4679
Peru balsam... 1.5730 to 1.5790
Persea gratissima (leaves).................................. 1.5139 to 1.5175
Pimento... 1.5303 to 1.5309
Pine needles.. 1.4725 to 1.4835

Rose (at 25°)... 1.4580 to 1.4650
Rosemary.. 1.4670 to 1.4735
Rue... 1.4300 to 1.4340

Santalwood.. 1.5050 to 1.5100
Sassafras... 1.5200 to 1.5300
Savin... 1.4730 to 1.4790
Saw palmetto.. 1.4120 to 1.4135
Snake root.. 1.4850 to 1.4900
  pearmint.. 1.4800 to 1.4970
  pike lavender... 1.4650 to 1.4675
Storax (Asia Minor)... 1.5395 to 1.4655
Sweet birch... 1.5350 to 1.5380

Thuja (leaf).. 1.4574 to 1.4585
Thyme... 1.4800 to 1.4900
Tolu balsam... 1.5440 to 1.4600
Turpentine.. 1.4690 to 1.4740

Vetivert.. 1.5150 to 1.5270

Wintergreen... 1.5350 to 1.5375

Witch-hazel.....	1.4830 to 1.4895
Wormseed (American)......................................	1.4740 to 1.4760
Wormwood...	1.4600 to 1.4710
Ylang-Ylang..	1.4780 to 1.5125
Zedoary	1.5020 to 1.5060

Alcohols of the Geraniol Series.

Geraniol.—In reference to the constitution of geraniol as given on page 258 of Vol. IV, it is still unsettled whether that formula is correct or whether geraniol should be represented as follows:

$$\begin{matrix} H_3C \\ H_2C \end{matrix} C .CH_2.CH_2.CH_2.C(CH_3) : CH.CH_2.OH$$

A corresponding alternative formula for the stereoisomer nerol (page 263, Vol. IV) naturally follows.

Citronellol.—An alternative formula for citronellol (see page 263, Vol. IV) has been suggested as follows:

$$\begin{matrix} H_3C \\ H_2C \end{matrix} C.CH_2.CH_2.CH_2.CH(CH_3).CH_2.CH_2.OH$$

The chemistry of citronellol is in a somewhat unsettled condition. Rupe[1] has clearly shown that citronellal exists in two modifications, and this may well be true of citronellol. Tiemann and Schmidt[2] held that *lævo*-citronellol from geranium and rose oils and *dextro*-citronellol obtained from citronellal are merely optically active isomers, whilst Barbier and Bouveault,[3] Bouveault and Gourmand,[4] and Barbier and Léser[5] maintain that they are two alcohols of different constitution. Barbier and Locquin[6] have now carried out a further series of experiments with a view to show that citronellol and rhodinol are in fact chemical isomers. They state that by attaching a halogen acid to *d*-citronellol or *l*-citronellol, treating the resulting compound with glacial acetic acid and sodium acetate, and subsequently hydrolysing, *d*-rhodinol or *l*-rhodinol results. They also claim that rhodinol on oxidation yields an aldehyde, rhodinal, which is not identical with citronellal.

Linalol.—The figures usually recorded for the sp. gr. of linalol are now known to be too high. Schimmel & Co. have prepared a pure specimen by decomposing linalol phenylurethane. Its sp. gr. at 15° was found to be 0.8666.[7] Tiemann[8] also found this to be the case, his pure specimen having a sp. gr. 0.8622 at 20°.

Nerol.—The view that geraniol (*q.v.*) and nerol are stereoisomers ex-

[1] *Annalen*, 1914, 402, 149.
[2] *Ber.*, 1896, 29, 903.
[3] *Compt. rend.*, 1896, 122, 737, 793.
[4] *Compt. rend.*, 1904, 138, 1699.
[5] *Compt. rend.*, 1897, 124, 1308.
[6] *Compt. rend.*, 1913, 157, 1114.
[7] *Report*, Oct., 1911, 141.
[8] *Ber.*, 1898, 31, 834.

pressed on pages 262 and 263 in Vol. IV has been confirmed by Blumann and Zeitschel.[1]

Nerolidol and Farnesol.—These two substances are not, chemically, in any close relationship to the geraniol alcohols, but, as it is not necessary to deal in this work with the sesquiterpene alcohols as a class, and as these bodies are found in the same group of oils, they may be dealt with conveniently in this section.

Nerolidol is an alcohol of the formula $C_{15}H_{26}O$ which is found in the high boiling fractions of neroli oil. Its odour is sweet, but not powerful, and it has recently been found to be the principal constituent of peruviol, an oil isolated from balsam of Peru by Thoms and believed by him to be a chemical individual.[2] Nerolidol boils at 276–277° or at 128–129° under 6 mm. and has a sp. gr. 0.880, $[\alpha]_D + 13°$, and ref. index 1.48023.

Farnesol is a sesquiterpene alcohol, having the formula $C_{15}H_{26}O$, which has been isolated from a number of the natural flower ottos. It has been identified in the essential oils of cassie flowers (*Acacia farnesiana*), rose, ylang-ylang and ambrette seeds. Its odour reminds one strongly of that peculiar odour which runs through all pure Bulgarian otto of rose, and known to experts as the "honey" odour—an odour somewhat recalling that of honeysuckle flowers. There is also in it a suggestion of a heavy odour, characteristic of the sesquiterpene alcohols, of the Oriental type, somewhat resembling that of cedar wood.

Farnesol is an oil boiling at 160° under 10 mm. pressure, and 149° under 4 mm. Its sp. gr. is about 0.890, and ref. index 1.4880. It is optically inactive. These characters indicate that it is more closely allied to the so-called olefinic sesquiterpenes than to the normal sesquiterpenes, which have a sp. gr. considerably over 0.900, and a ref. index of 1.500 or more.

Farnesol may be characterised by converting it by oxidation with chromic acid as described under the oxidation of geraniol, when it yields an aldehyde, which has been termed *farnesal*. This compound yields a semi-carbazone in the usual manner, m. p. 133°–135°.

Cyclic Terpene Alcohols.

Borneol.—The highest rotatory power observed for a natural borneol is $[\alpha]_D - 39° 25'$ (*Schimmel's Report*, April, 1895, 74). To differentiate between borneol and isoborneol, Henderson and Heilbron[3] recommend preparing the nitrobenzoates of the alcohols. The alcohol, dissolved in from 10 to 15 times its weight of pyridine is heated on the water-bath with about the calculated quantity of *p*-nitrobenzoyl chloride. The pyridine is extracted with diluted sulphuric acid, at 0°, and the *p*-nitrobenzoate washed with

[1] *Ber.*, 1911, **44**, 2591.
[2] *Schimmel's Report*, April, 1914, **80**.
[3] *Proc. Chem. Soc.*, 1913, 381.

dilute sulphuric acid, dried, and recrystallised from alcohol. The borneol compound melts at 137° and the isoborneol compound at 129°.

Fenchyl Alcohol.—This alcohol forms a phthalic acid ester, m. p. 145°, and phenylurethane, m. p. 82–83°.

Pickard, Lewcock and Yates[1] have prepared pure *lævo*-fenchyl alcohol, by reducing fenchone and converting the fenchyl alcohol into the hydrogen phthalate. By fractional crystallisation of the magnesium and cinchonine salts, a hydrogen phthalate was obtained, which, on saponification gave *lævo*-fenchyl alcohol $[\alpha]_D - 15° 30'$.

Thujyl Alcohol.—For the isomeric forms of thujyl alcohol reference should be made to a paper by Paolini.[2]

Terpineol.—The liquid terpineol of commerce manufactured from terpin hydrate by the action of dilute sulphuric acid consists of a mixture of *a*-terpineol, m. p. 35°, which predominates, and of β-terpineol m. p. 32° (incorrectly described as *p*-terpineol on page 281 of Vol. IV, line 2), and of liquid terpineol[3] m. p. — 1°. The terpineol melting at 69–70° has been termed γ-terpineol. The three varieties have the following characters:

	α	β	γ
M. p.	35°–38°	32°–33°	69°–70°
Sp. gr.	0.935–0.940	0.923	
Rotation	up to − 117°		
Ref. index	1.4810–1.4827	1.4747	
B. p.	217–219°	210°	

Menthol.—Absolutely pure menthol melts, according to Schimmel & Co. (*Die Ätherischen Öle*, Band I, p. 405) at 43.5° to 44.5°. The specific rotatory power of melted menthol at 46° is −50°, and in a 20% alcoholic solution at 20°, is −49.35°. For the characters of *iso*menthol see Pickard and Littlebury.[4]

Cineol.—This neutral substance is incorrectly classed as an alcohol on page 284 of Vol. IV.

Alcohol	Source	M. p.	B. p.	Rotation $[\alpha]_D$	Ref. index	Sp. gr.
Amyrol	W. Indian santal oil	liquid	300°			
Atractylol	Atractylis oil	59°	292°	0°	1.5110	
Betulol	Betula oil	liquid	293°	− 35°	1.5018	0.975
Cedrol	Cedarwood oil	86–87°	294°	+ 9.5°	1.5139	1.0056 (at 20°)
Cubeb-camphor	Cubeb oil	68–70°	248°			
Eudesmol	Eucalyptus oil	88°	284°	−35.5°	1.5164	0.9884
Farnesol	Flower oils	liquid	160° (10 mm.)	0°	1.4881	0.887
Guaiol	Bulnesia oil	91°	288°	− 30°	1.5100	0.971
Ledum-camphor	Marsh tea oil	104°	281°		1.5072	0.9814
Nerolidol	Neroli oil	liquid	277°	+13.5°		
Patchouli-camphor	Patchouli oil	56°	296°	−97.6°	1.5245	1.028
a-Santalol	Santal oil	liquid	301°	− 1.2°		0.977
β-Santalol	Santal oil	liquid	310°	− 56°		0.9784

[1] *Proc. Chem. Soc.*, 1913, **29**, 127.
[2] *Rend. R. Acad. Lincei*, **20**, i, 765.
[3] Wallach, *Liebig's Annalen*, 1907, **356**, 218; 1908, **362**, 342.
[4] *Trans.*, 1912, 1019, 10.

Sesquiterpene Alcohols.

The preceding table brings the principal characters of the sesquiterpene alcohols up to date.

Aldehydes of the Geraniol Series.

Citral.—It is uncertain whether citral has the constitution assigned to it on page 267 of Vol. IV, or whether it is represented by the following formula:

$$CH_3 \diagdown C.CH_2.CH_2.CH_2.C(CH_3) : CH.CHO$$
$$CH_3 \diagup$$
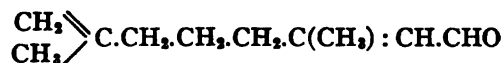
Citronellal.—Rupe[1] has shown that there exist two isomeric forms of citronellal.

ERRATA IN VOL. IV.

Pages 180 and 315, Pinene does not form a constituent of oil of bay and its presence in such oil is a sign of adulteration.

Page 182, line 12, for "derivations" read "derivatives."

Page 189, line 12 from bottom, for " Thorns" read " Thoms."

Page 210, line 1 of footnote, for " Thomas" read " Thoms."

Page 233, line 17, for "Cassio" read "Cassia" and add "See Vol. III, p. 442."

Page 224, in Table, for "Anethol" read "Anethole."

Page 281, line 2, for "p-terpineol" read "β-terpineol."

[1] *Annalen*, 1914, 402, 149.

SPECIAL CHARACTERS OF ESSENTIAL OILS.

By ERNEST J. PARRY, B.Sc., F.I.C.

ANDROPOGON OILS.

Most of these oils are now referred to the genus *Cymbopogon* which was formerly recognized only as a subgenus.

Citronella Oil.—The parent plant of citronella grass is known as mana grass of which Stapf distinguishes two varieties, *Cymbopogon Nardus, var. Linnæi*, and *C. Nardus var. confertiflorus*. The natives differentiate still further in Ceylon and the following table illustrates this differentiation, with the character of the various oils themselves.

No.	Parent Plant		d_{15}	α_D	Total alcohol %	Geraniol %	Citronellal %
	Botanical name (According to Stapf's determination)	Native name					
1	*C. Nardus*, Rendle var. *Linnæi*, Stapf (*typicus*)	Maha-naran-pengiri	0.920	− 3° 7′	51.6	27.6	24.0
2	*C. Nardus*, Rendle var. *Linnæi*, Stapf (*typicus*)	Maha-naran-pengiri	0.905	− 6°32′	63.2	38.4	24.8
3	*C. Nardus*, Rendle var. *Linnæi*, Stapf (*typicus*)	Maha-naran-pengiri	0.912	+ 3° 22′	57.2	36.2	21.0
4	*C. Nardus*, Rendle var. *Linnæi*, Stapf (*typicus*)	Heen-naran-pengiri	0.913	+ 2° 35′	43.5	24.6	18.9
5	*C. Nardus*, Rendle var. *Linnæi*, Stapf (*typicus*)	Heen-naran-pengiri	0.894	− 3° 20′	47.7	25.8	21.9
6	*C. Nardus*, Rendle var. *Linnæi*, Stapf (*typicus*)	Heen-naran-pengiri	0.909	+ 2° 6′	53.5	30.0	23.5
7	*C. Nardus*, Rendle var. *Linnæi*, Stapf (*typicus*)	Light-leaved mana	0.909	+ 4° 54′	56.5	38.6	17.9
8	*C. Nardus*, Rendle var. *Linnæi*, Stapf (*typicus*)	Light-leaved mana	0.908	+ 3° 30′	64.0	30.2	33.8
9	*C. Nardus*, Rendle var. *Linnæi*, Stapf (*typicus*)	Small-leaved mana	0.906	+ 3° 7′	57.0	34.4	22.6
10	*C. Nardus*, Rendle var. *Linnæi*, Stapf (*typicus*)	Small-leaved mana	0.909	+ 3° 20′	56.3	36.5	19.8
11	*C. Nardus*, Rendle var. *Linnæi*, Stapf (*typicus*)	Sour mana	0.935	+16°	35.3	16.3	19.0
12	*C. Nardus*, Rendle var. *Linnæi*, Stapf (*typicus*)	Sour mana	0.967	+15°50′	26.9	6.3	20.6
13	*C. Nardus*, Rendle var. *Linnæi*, Stapf (*typicus*)	Very broad-leaved mana	0.926	+ 1°31′	48.7	25.8	22.9
14	*C. Nardus*, Rendle var. *Linnæi*, Stapf (*typicus*)	Very broad-leaved mana	0.906	− 0° 24′	64.7	36.5	28.2
15	*C. Nardus*, Rendle var. *Linnæi*, Stapf (*typicus*)	Given as "maha-pengiri," but probably "mana"	0.912	− 1° 38′	48.6	25.5	23.1
16	*C. Nardus*, Rendle var. *Linnæi*, Stapf (*typicus*)	Given as "maha-pengiri," but probably "mana"	0.909	− 2°18′	56.4	35.8	20.6
17	*C. Nardus*, Rendle var. *confertiflorus*, Stapf	Glaucous-leaved mana	0.913	+12° 12′	46.5	29.3	17.2

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No.	Parent Plant		dis	a_D	Total alcohol %	Geraniol %	Citronellal %
	Botanical name (According to Stapf's determination)	Native name					
18	C. *Nardus,* Rendle var, *confertiflorus,* Stapf	Glaucous-leaved-mana	0.900	+ 4°	61.2	43.7	17.5
19	C. *Nardus,* Rendle var. *confertiflorus,* Stapf	White-stemmed mana	0.908	+ 1° 27'	54.8	30.2	24.6
20	C. *Nardus,* Rendle var. *confertiflorus,* Stapf	White-stemmed mana	0.904	+ 2° 26'	58.0	24.8	33.2
21	C. *Nardus,* Rendle var. *confertiflorus,* Stapf	Red-stemmed mana	0.929	+ 6° 19'	39.1	19.4	19.7
22	C. *Nardus,* Rendle var. *confertiflorus,* Stapf	Red-stemmed mana	0.909	+ 0° 58'	57.0	28.9	28.1
23	C. *Nardus,* Rendle, closely allied to var. *confertiflorus*	Lenabatu-pengiri (not genuine)	0.915	+ 2° 46'	52.0	31.1	20.9
24	C. *Nardus,* Rendle, closely allied to var. *confertiflorus*	Lenabatu-pengiri (not genuine)	0.902	— 2° 11'	63.1	39.5	23.6
25	C. *Nardus,* Rendle, closely allied to var. *confertiflorus*	Lenabatu-pengiri (not genuine)	0.907	— 0° 6'	64.2	44.8	19.4

The Maha-pengiri grass, which is cultivated largely in Java is *Cymbopogon Winterianus.*

The constituents of Ceylon citronella oil hitherto identified are as follows: citronellal, geraniol, camphene, dipentene, methyl-heptenone, borneol, methyleugenol, limonene, thujyl alcohol (?), nerol, geranyl acetate, *d*-citronellyl acetate and butyrate and a sesquiterpene of sp. gr. 0.8643, probably identical with an aliphatic sesquiterpene isolated by Semmler and Spornitz[1] from Java citronella oil, and which has the following characters: sp. gr. 0.8489, optical rotation +0° 36', ref. index 1.5325, and b. p. 138–140° at 9 mm. pressure. Elze[2] has quite recently isolated about 0.25% of farnesol from this oil. The Java oil contains geraniol, citronellal, *d*-citronellol, traces of methyleugenol, citral, isovaleric aldehyde, *iso*-amyl-alcohol and the sesquiterpene, citronellene, above mentioned. The following figures may be taken to cover the vast majority of genuine samples. But it must be remembered that practically the whole of the oil exported in drums from Ceylon is adulterated with petroleum—so as just to pass Schimmel's test. The custom of purchasing on a geraniol standard (*i.e.*, total acetylisable constituents) is rapidly gaining ground.

.	Ceylon oil	Java oil
Sp. Gr..........................	0.898 to 0.920	0.884 to 0.900
Optical rotation.................	—7° to —20°	0° to — 3°
Ref. index at 20°................	1.4790 to 1.4890	1.4650 to 1.4720
Total "Geraniol".................	57 % to 64%	83 % to 96%

Lemon-grass Oil.—Formerly it was true that lemon-grass oil distilled in the East was soluble in 3 volumes of 70% alcohol, whereas that distilled in the West Indies, although soluble when freshly distilled rapidly lost its solubility. This is not, however, true today, and nearly all lemon-grass oil

[1] *Ber.,* 1913, **46,** 4025.
[2] *Chem. Zeil.,* 1913, **37,** 1422.

is at the present time insoluble in 3 volumes of 70% alcohol. Further, on the voyage from the East it steadily loses its citral strength and samples examined a year after distillation will frequently show a loss of 5 to 8% in citral. Whether this is due to the use of different types of grass, or different method of cultivation is not yet understood.

Numerous samples of lemon-grass oil from various parts of the world have recently been examined, such as Uganda, Bermuda, Montserrat, Seychelles, Sumatra, Mayotte and Burmah. These, however, are scarcely commercial articles and call for no remarks here.

Palmarosa Oil and Ginger-grass Oil.—The grass known as *Cymbopogon Martini* exists in two varieties, the native names being "motia" and "sofia." These names have been adopted by Burkill, and the grasses are known as follows:

Cymbopogon Martini var. Motia = palmarosa oil
Cymbopogon Martini var. Sofia = ginger-grass oil.

The characters of the two oils are as follows:

	Palmarosa	Ginger-grass
Sp. Gr.	0.886 to 0.900	0.900 to 0.953
Optical rotation	+6° to −3°	+54° to −30°
Ref. index	1.4720 to 1.4760	1.4780 to 1.4930
Acid value	0.5 to 3.0	to 6.2
Ester value	12 to 48	8 to 29 (rarely to 54)
Total geraniol	75 to 95 %	35 to 65 %
Solubility in 70 % alcohol	1 in 1.5 to 3 volumes	1 in 2 to 3 volumes

Traces of farnesol have been found in palmarosa oil,[1] and also traces of caproic acid in the form of esters.

Ginger-grass oil contains d-α-phellandrene, dipentene, d-limonene, heptaldehyde, citronellal or an isomeric aldehyde, i-carvone, geraniol and dihydrocumic alcohol.

Vetivert Oil.—This oil is distilled from the roots of *Vetiveria zizanioides*, formerly known as *Andropogon muricata*. It is a viscous oil with a heavy, penetrating odour, and is used to a considerable extent in the manufacture of perfumes of the oriental type. The roots are known in India by the name of Khas-Khas or cus-cus.

Genvresse and Langlois isolated from this oil a hydrocarbon $C_{15}H_{24}$ which they termed *vetivene* and an alcohol, *vetivenol*, $C_{15}H_{26}O$ and an ester of this alcohol and an acid of the formula $C_{15}H_{24}O_2$. Semmler and his colleagues[3] have more recently shown that vetivenol has the formula $C_{15}H_{24}O$, and the acid $C_{15}H_{22}O_2$. For the differences between the alcohol and esters present in vetivert oils of different origins, the original paper should be consulted.

[1] Else, *Chem. Zeit.*, 1910, 34, 857.
[2] *Compt. rend.*, 1902, 135, 1059.
[3] *Ber.*, 1912, 45, 2347.

The oil distilled in Europe from the dried imported root has the following characters:

Sp. gr	1.015 to 1.040
Optical rotation	+25° to +37°
Ref. index to 20°	1.5220 to 1.5270
Acid value	27 to 65
Ester value	9.8 to 23
Ester value after acetylation	130 to 158

It is soluble in 1 to 2 volumes of 80% alcohol with turbidity.

The oil distilled in Réunion from the fresh roots has the following characters:

Sp. gr	0.980 to 1.020
Optical rotation	+22° to +37°
Ref. index	1.5150 to 1.5270
Acid value	4.5 to 17
Ester value	5 to 20
Ester value after acetylation	124 to 145

It is soluble in 1 to 2 volumes of 80% alcohol with turbidity.

Fiji and Seychelles vetivert oils do not differ materially from the above described.

Puran Singh[1] has examined the oil distilled in India from Indian-grown roots, and his results suggest that in the distillation of the roots a small amount of resin is carried over, and that if the oil be freed from this by careful redistillation, the resulting oil is lævorotatory. 75 grm. of oil were carefully steam distilled and about 65 grm. of oil resulted. The residue was a dark-red resinous mass, which was extracted with alcohol. About 9.4 grm. of a dark-red resin of a brilliant fracture were obtained. It softened at 65°, and completely melted at 70°. It gave the following constants:

Sp. gr. at 30°	1.132
Acid number	46.92
Saponification number	111.02
Ester number	64.28
Iodine value (Hübl, after 18 hours)	268.20
Optical rotation calculated on 10 c.c. of the solid resin	+488.04

The redistilled oil, which was of a yellowish-brown colour and quite transparent, gave the following constants:

Sp. gr. at 15°	1.011
Optical rotation in 100-mm. tube	−30.65
N_D 20°	1.5165
Acid number	10.5
Saponification number	80.1
Ester number	69.6
Saponification number after acetylisation	132.8
Iodine value (Hübl, after 18 hours)	194.4
Soluble in 2 parts of 80% alcohol.	

The above constants are different from those already recorded by various observers. This difference may be due to the elimination of the resin from the oil by redistillation; at least in optical rotation it is mainly due to this cause. The angle of rotation of the oil in this case is about −31°, while all observers have recorded for vetivert oil a rotation of +25° to +40°. The optical rotation of the resin is +488.4°. Taking the proportion of the oil to

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[1] *Chem. and Druggist,* 1914, 2, 51.
[2] Approximately; because the solution had to be made too dilute for observation, owing to the very dark colour of the resin.

resin, as it is in this case, to be 8 : 1, the optical rotation calculated on the mixture of resin and oil comes to +34°. It seems that the dextrorotation of the oil is due to the small proportion of *Khas-Khas* resin. This will also have its effect on other constants. It is not known whether the constants of this oil as recorded by other observers were determined on redistilled oil or on the first distillate. If on the latter, then the constants of this resin-free oil will in all cases be different from those already published. This has yet to be confirmed.

Almond Oil.

Essential oil of bitter almonds is a commercial product of considerable importance, as it is used to a large extent in the preparation of flavouring essences.

The true bitter almond oil is obtained by distillation from the seeds of *Prunus amygdalis* (*Amygdalis communis*), the ordinary bitter almond. But as the kernels of the peach and apricot yield an oil which is indistinguishable from that of the almond, it is certain that much of the almond oil of commerce is really derived from peach and apricot kernels. Hence the remarks made here apply to all three oils indiscriminately.

The oil does not exist as such in the seeds but is produced by the action of the enzyme emulsin on the glucoside amygdalin, under the influence of water. Without discussing the question of intermediate products, the action taking place results, in the main, in the formation of benzaldehyde and hydrocyanic acid as follows:

$$C_{20}H_{27}NO_{11} + 2H_2O = C_7H_6O + HCN + 2C_6H_{12}O_6$$

amygdalin   benzaldehyde   glucose

The natural oil therefore contains hydrocyanic acid and is highly poisonous. The commercial article is therefore usually deprived of hydrocyanic acid and sold as "Ol. amygdalæ essent. sine acido prussico (S.A.P.)."

Composition.—This oil consists principally of benzaldehyde (with or without hydrocyanic acid) and a little benzaldehyde-cyanhydrin, $C_5H_6(CH)(OH)CN$ formed by the interaction of the aldehyde and the acid.

Characters.—The sp. gr. of the natural oil varies between 1.045 and 1.070. It is optically inactive or at most, very faintly dextrorotatory, up to +0° 10'. Its ref. index is from 1.5320 to 1.5440.

Hydrocyanic acid is detected by shaking the oil with water, and then adding to the water minute quantities of ferrous and ferric chlorides, and then solution of caustic potash. On adding hydrochloric acid a blue colour or precipitate is formed if hydrocyanic acid be present. To estimate the hydrocyanic acid, 1 grm. should be dissolved in 5 c.c. of alcohol and 50 c.c of water added. An ammoniacal solution of silver nitrate is then added, and the whole well shaken. The liquid is then acidified with nitric acid, and the silver

cyanide is collected, washed and weighed as silver after ignition.   Four parts of silver correspond to one of hydrocyanic acid.

If deprived of hydrocyanic acid, the oil has a sp. gr. 1.050 to 1.055, and a ref. index of 1.5420 to 1.5460 at 20°.   It boils principally at 179° and is soluble in 1 to 2 volumes of 70% alcohol.   Nitrobenzene has been found as an adulterant of this oil, but is rarely used now.   Its sp. gr. is about 1.200 and its odour is characteristically coarse.   It can be detected with certainty by boiling the oil with a little acetic acid and iron filings.   The nitrobenzene is reduced to aniline, which is distilled off and a few drops of chlorinated lime solution added to the distillate; the characteristic violet colour results if aniline be present.   The usual adulterant, however, is synthetic benzaldehyde.   This can be detected, chemically, only if it contains traces of chlorine, which the lower grades do, having been made by a condensation process in which hydrochloric acid is used.   To detect chlorine, a pure filter paper is saturated with the oil and placed in a small porcelain dish, standing in a larger one, and a beaker whose sides are moistened with distilled water is inverted over the smaller dish after the paper has been lighted.   The beaker is washed out with a few drops of distilled water, and the liquid filtered, and the filtrate tested for chlorides in the usual manner.   Heyl[1] recommends the following method:

1 to 2 grm. of chlorine-free calcium hydroxide are stirred up with a glass rod in a small porcelain dish with 10 to 15 drops of benzaldehyde, the mixture is then covered with a thin layer of calcium hydroxide and slowly brought to a red heat.   After cooling, the contents of the dish are poured into a beaker, 5 to 6 c.c. of water are carefully added, and the mixture is faintly acidified with nitric acid.   The solution is then filtered through chlorine-free filtering paper or glass-wool and tested for chlorine with silver nitrate.   Rupp[2] prefers the following test:

A copper wire, or, better still, a strip of copper wire-netting 0.5 cm. in width (about 1 mm. mesh) is twisted at one end into a narrow spiral shape, forming a roll about the diameter of a large pea.   This roll is repeatedly drawn through a non-luminous flame in order to oxidise the surface of the copper and to remove any yellow or green colouration.   When cool, the roll is dipped into the benzaldehyde under examination and is then exposed to the flame for a moment in order to inflame the benzaldehyde.   When the benzaldehyde is burnt off (not in the flame) the roll is again applied to the top of the flame.   If there is a green colouration, chlorine is present.

Angelica Oil.

Schimmel & Co.[3] give the following values for angelica oil distilled by themselves from various parts of the plant.

[1] Apoth. Zeit., 1912, 27, 49.
[2] Apoth. Zeit., 1912, 27, 49.
[3] Report, April, 1911, 20.

Oil from	Sp. gr. at 15°	Rotation	Ref. index at 20°.	Acid No.	Ester No.
Leaves...............	0.8550	+28° 2′	1.4778	0.5	17.6
Leaves...............	0.8697	+22° 8′	1.4804	1.3	22.6
Leaves...............	0.8767	+20° 11′	1.4832	1.6	21.1
Root.................	0.8733	+28° 23′	1.4808	2.5	25.9
Seed.................	0.8623	+12° 12′	1.4868	1.1	18.1

Aniseed Oil.

Schimmel & Co.[1] have identified the following terpenes in oil of star aniseed: d-α-pinene; d-β-phellandrene; dipentene; l-limonene.

Contrary to the statement made on page 312 of Vol. IV, the solidifying point of aniseed oil is a factor of the highest importance. The fact that the oil can exist in the liquid condition at a temperature much below its congealing point is immaterial, since crystallisation can be induced by sowing the oil with a crystal of anethole, or by skilful stirring with the thermometer. If the oil, in a narrow test-tube, be cooled to about 9° or 10°, and a crystal of anethole added, or crystallisation induced by stirring, the thermometer will rapidly rise as crystallisation sets in, and the maximum temperature, which a little experience will readily determine, indicates the true congealing point. In the best oils this will be at least 15°, and congealing points below this indicate inferior oils, or oils from which anethole has been deliberately extracted.

The presence of petroleum oil in aniseed oil is very easily detected by the lowering of the sp. gr., the lowering of the melting point and ref. index, and the insolubility in even large quantities of 90% alcohol, when petroleum oil separates and can be examined and identified.

A number of samples have of recent years been found on the market which have either been adulterated with such an oil as camphor oil or from which large quantities of anethole have been abstracted; see Parry,[2] Umney[3], Jensen[4] and Durrans.[5] It is still a question of doubt as to which form of adulteration had been adopted, but Parry recommends dividing the oil into the following fractions: the first = 10%, the three following = 25% each, and the residue = 15%. The following tables show the melting point and ref. index of a normal oil and one suspected by Parry of being adulterated by the addition of such an oil as heavy camphor oil, and the same figures for a normal oil and an oil from which anethole had purposely been abstracted, as illustrating Messrs. Schimmel & Co.'s views that the adulteration has been due to abstraction of anethole.

The figures in the following tables for normal oils will be found useful in judging aniseed oils which are at all doubtful, apart from whether the adulteration is by addition of a foreign oil or by the abstraction of anethole.

[1] *Report*, October, 1911, 86.
[2] *Chemist and Druggist*, 1910, 77, 687.
[3] *Perf. and Ess. Oil Record*, 1910, 1, 236.
[4] *Pharm. J.*, 1910, 85, 759.
[5] *Perf. and Ess. Oil Record*, 1911, 2, 60.

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Nr.	Amount of fraction	Normal star anise oil (m. p. 18°)		Suspected star anise oil (m. p. 12.5°)	
		M. p.	$n_{D_{20}}$ to 21°	M. p.	$n_{D_{20}}$ to 21°
1	10 %	8°	1.5316	−3°	1.5125
2	25 %	18°	1.5500	15°	1.5419
3	25 %	20°	1.5540	17.5°	1.5500
4	25 %	20°	1.5591	18°	1.5521
5 (Residue)	15 %	15°	1.5522	11°	1.5467

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Nr.	Amount of fraction	Normal star anise oil (sol. p. 16.5°)		The same oil after abstraction of part of its anethole (sol. p. 10.1°)	
		Sol. p.	$n_{D_{20}}$°	Sol. p.	$n_{D_{20}}$°
1	10 %	7.5°	1.53279	under 0°	1.50326
2	25 %	15.7°	1.55125	8.1°	1.53885
3	25 %	18.0°	1.55866	14.5°	1.55342
4	25 %	19.4°	1.55980	15.2°	1.55723
5 (Residue)	15 %	7.5°	1.56079	under 0°	1.55505

Bay Oil.—The oil from Californian Bay (Vol. IV, p. 316) has the following characters:

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Sp. gr.................................................. 0.930 to 0.950
Optical rotation........................................ −22° to −24°
Acid value.............................................    about 5
Ester value............................................    about 5
Ester value after acetylation..........................    about 50
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It is soluble in 1.5 to 2.5 volumes of 70% alcohol. This oil contains eugenol, l-α-pinene, safrole, methyl-eugenol, and about 40 to 60% of *umbellulone*, a ketone of sp. gr. 0.950 at 20°, optical rotation − 36° 30′, ref. index 1.48325, and b. p. 219–220°. It forms a semi-carbazone melting at 240°–243°.[1]

Bergamot Oil.—The principal adulteration of Bergamot oil today is by the addition of artificial esters together with a neutral body such as lemon terpenes. The detection of these is fully described under "*Esters*" (on pages 330–336). A pure bergamot oil should have the following characters:

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Sp. gr.................................................. 0.881 to 0.8865
Optical rotation........................................ +8° to +24°
Ref. index at 20°....................................... 1.4650 to 1.4675
Fixed residue..........................................    4.3 to 6 %
Sap. value of residue..................................    160 to 200
Acid value.............................................    1.0 to 3.8
Esters as linalyl acetate..............................    34 to 43 %
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The differences observed in the process of fractional saponification are given under "Esters" on page 331.

[1] Power and Lees, *Trans.*, 1904, 85, 629; also 1906, 89, 1104; 1907, 91, 271 and 1908, 93, 252.

Buchu Oil.

The oil distilled from buchu leaves is employed to some extent in medicine. The species usually found in commerce are *Barosma betulina*, *B. crenulata*, and *B. serratifolia*.

Diosphenol, $C_{10}H_{16}O_2$, is the most characteristic constituent of the oil, occurring to a considerable extent in that from *B. betulina*, whilst only to a small extent in that of *B. serratifolia*. It is a crystalline solid, m. p. 81° and b. p. 232°. It is a cyclic keto-phenol and is also known as buchu-camphor. The tepenes, limonene and dipentene are present and also *l*-menthone. The oils of the three species above mentioned have the following characters:

	B. betulina	B. crenulata	B. serratifolia
Sp. gr.	0.937–0.970	0.9364	0.918–0.961
Rotation	−14° to −18°	−15° 22′	−12° to −36°
Ref. index	1.4740 to 1.4870	1.4801	....

The oils from *Barosma pulchella* and *B. venusta* have also been examined, but are not commercial articles.[1]

Calamus Oil.

This oil is used, especially on the continent, as a flavouring material in the preparation of certain types of beers, liqueurs, etc. The European oil is distilled from the rhizome of *Acorus calamus*, whilst the Japanese oil is probably derived from *Acorus spurius*.

Russian calamus oil contains α-pinene, camphor, a sesquiterpene, calamene (having a sp. gr. 0.922, optical rotation +5° and ref. index 1.5057) and an alcohol $C_{15}H_{24}O$, which is termed *calamenenol*.[2] Traces of eugenol and heptylic acid are also present. Japanese calamus oil contains a small amount of methyl-eugenol. European calamus oil has a sp. gr. 0.958 to 0.970, optical rotation +9° to +35°; ref. index 1.5028 to 1.5078, acid value 1 to 2.5; ester value 5 to 20, and ester value after acetylation 32 to 50. It is soluble in practically any quantity of 90% alcohol. Japanese calamus oil has a sp. gr. 0.985 to 1.000, optical rotation +7° to + 25°, ester value about 4, ester value after acetylation, 17.

Camphor Oil.

The following are the constituents of *normal* camphor oil, only a limited number of which are present in the fractions known commercially as camphor oil.

[1] *Schimmel's Report*, April, 1909, 96 and April, 1910, 17; *Pharm. J.*, 1913, 90, 60.
[2] Semmeler and Spornitz, *Ber.*, 1913, 46, 3700.

(1) Acetaldehyde.
(2) d-α-pinene.
(3) Camphene.
(4) d-fenchene.
(5) β-pinene.
(6) Phellandrene.
(7) Cineol.
(8) Dipentene.
(9) d-limonene.
(10) Borneol.
(11) Camphor.
(12) Terpinenol.

(13) α-terpineol.
(14) Citronellol.
(15) Safrole.
(16) Δ'-menthenone-3.
(17) Carvacrol.
(18) Cumic alcohol.
(19) Eugenol.
(20) Bisabolene (sesquiterpene).
(21) Cadinene.
(22) Caprylic acid.
(23) An acid $C_9H_{14}O_2$.
(24) A blue oil.

In addition to the above bodies, which are given in the order of their boiling points, Semmler and Rosenberg[1] have isolated a sesquiterpene which they term sesquicamphene (sp. gr. 0.9015 at 20°, optical rotation + 3°, and refractive index 1.5006), and a sesquiterpene alcohol, which they name *sesquicamphenol*. A diterpene, $C_{20}H_{32}$, which has been named *α-camphorene*, of sp. gr. 0.8870 at 20° and b. p. 178° under 6 mm., was also isolated.

Crude camphor oil has a sp. gr. 0.950 to 0.995, and is usually fractionated after removal of most of the camphor, in Japan, into white camphor oil and "red" or "black" camphor oil. White camphor oil has a sp. gr. 0.870 to 0.910, and black camphor oil from 0.990 to 1.036.

Cardamon Oil.

Cardamom oil is obtained from the seeds of several varieties of cardamoms. Of these the principal are those known as Ceylon-Malabars and Ceylon-Mysores, both being varieties of *Elettaria cardamomum*. The fruit known as "Ceylon wilds" is probably derived also from a variety of the same plant. Siam cardamom is the fruit of *Amomum Cardamomum*. Terpinyl acetate is an important constituent of the oil, as well as free terpineol and cineol. The following are the characters of the various oils:

	Elettaria cardamomum	Celon wilds	*Amomum cardamomum*
Sp. gr.	0.923–0.944	0.895–0.906	0.905 at 42°
Rotation	+24° to +47°	+12° to +15°	+38°
Ref. index	1.4620–1.4670		
Acid value	to 4		0.8
Ester value	94–150	– 25–70	14–18.8
Solubility in 70 % alcohol	1 in 2 to 5 vols.		

This oil is used to a certain extent in flavouring cakes, sauces and other food products.

Cassia and Cinnamon Oils.

Cassia oil is an oil largely employed in the perfuming of cheap soaps, etc. It is distilled from the leaves, twigs and other parts of *Cinnamomum cassia*, a native of Cochin China. The cinnamon oil of commerce is obtained from the bark of *Cinnamomum zeylanicum*, a native of Ceylon. The leaves of this tree also yield an essential oil, which is a commercial article.

[1] *Ber.*, 1913, 46, 768.

Cassia Oil.—The principal constituent of this oil is cinnamic aldehyde, small quantities of a terpene, cinnamic esters and o-methyl-coumaric aldehyde being also present. It is customary to grade cassia oils on the market as 70–75, 75–80, and 80–85%, these values indicating the cinnamic aldehyde content. But as pure oils may, and probably usually do, contain from 85 to 90% or even more, it has become a recognised custom to standardise the lower grade oils with resin, so that a 70–75% oil will usually contain 71%, and 80–85% oil 81% of cinnamic aldehyde.

A pure cassia oil has a sp. gr. 1.055 to 1.070, optical rotation −1° to +6°, ref. index, 1.6020 to 1.6075, and acid value 6 to 15. It is easily soluble in 2 volumes of 80% alcohol.

The cinnamic aldehyde should be determined in a Hirschsohn flask, using 5 c.c. of oil and a hot 30–35% solution of sodium hydrogen sulphite. The flask is kept in a boiling water-bath until the whole of the crystals formed are dissolved, the flask being well shaken at frequent intervals. The unabsorbed oil is then forced into the neck of the flask by adding more of the solution and when cold, the oil is read off, the difference being calculated as cinnamic aldehyde. It is probable that this process gives slightly too high results, whilst the use of neutral sodium sulphite gives results about 3 to 4% lower. It has become an established trade custom, however, to return all results for this oil by the bisulphite process.

The influence of added resin is to raise the acid value of the oil considerably and to cause the oil to leave a high distillation residue. Cassia oils free from resin do not give appreciable precipitates with a saturated solution of lead acetate in alcohol.

The following is the best method of using the lead acetate test, and from the figures quoted[1] it will be seen that the higher the lead precipitate, the higher the acid value, and consequent percentage of added resin.

5 grm. of the oil are dissolved in 20 c.c. of 70% alcohol and 10 c.c. (or more if necessary) of a saturated solution of lead acetate are added. The precipitate is collected on a tared Gooch filter (packed with ignited asbestos) and thoroughly washed with 70% alcohol. The filtrate should be tested with more of the lead acetate solution and any further precipitate filtered off. The precipitate is dried at 100° to constant weight. Results obtained on prepared oils were as follows:

    1. Cassia oil, containing 10 % resin gave 10.6 % lead ppt.
    2. Cassia oil, containing 15 % resin gave 15.2 % lead ppt.
    3. Cassia oil, containing 20 % resin gave 19.7 % lead ppt.

The determination of the acid value of cassia oil, suggested by Yates,[2] cannot be relied upon to indicate exactly the proportion of added resin, on account of the natural variation in the acid values of the oil and resin respectively, and one should hesitate to place reliance on this factor as a means

[1] *Perf. and Ess. Oil Record*, 1914, **7**, 264.
[2] *Perf. and Ess. Oil Record*, 1912, page 83.

of judging the percentage of added resin, although it is significant that oils with a high aldehyde content have low acid values.

The following are typical examples of the results obtained:

	Aldehyde content	Acid value	Lead ppt.
1. Cassia oil free from resin.................	92	6	nil.
2. Cassia oil free from resin.................	88	13	nil.
3. Cassia oil containing resin..............	82	27	10.1
4. Cassia oil containing resin..............	73	29	10.1
5. Cassia oil containing resin..............	72	23	10.6

An approximate determination of added resin may be obtained by distilling 50 grm. in a small tared Wartz flask and stopping the distillation when the temperature reaches 280°. The residue should not exceed 10%, rarely over 8%, so that any excess may be regarded as resin.

Cinnamon Bark Oil.—Although the greater part of the world's supply of this oil comes from Ceylon, a certain amount is distilled, on a small scale in other countries, and Seychelles cinnamon oil has recently been carefully examined. Cinnamic aldehyde is the constituent present in largest quantity, but as this oil contains less aldehyde than cassia oil, whilst its odour is far sweeter and more delicate, it is obvious that much of the perfume value lies in the non-aldehydic portion of the oil. The oil contains, in addition, eugenol, methyl-amyl ketone, benzaldehyde, pinene, phellandrene, cymene, nonylaldehyde, cumic aldehyde, hydrocinnamic aldehyde, linalol, linalyl esters, and caryophyllene.

For slight differences in the composition of Seychelles cinnamon oil see Schimmel's Report, April, 1913, page 42. According to most authorities pure cinnamon oil has a sp. gr. 1.020 to 1.040, but Hill[1] and Umney and Bennett[2] consider that a lower sp. gr., from 0.994 to 1.022 is normal for pure cinnamon oil. Schimmel & Co. consider that these low sp. gr. are due to abnormal distillation, a certain amount of the aldehyde being oxidised and lost in the distillation. The oil is faintly lævorotatory, up to −1°, and has a ref. index 1.5810 to 1.5910. It contains from 55 to 65% of cinnamic aldehyde (or according to Hill, Umney and Bennett, *loc. cit.*, between 50 and 60%); it should not contain more than 5–10% (rarely more than 6%) of eugenol, as determined by absorption with 5% potassium hydroxide solution. It is soluble in 2 to 3 volumes of 70% alcohol. The principal adulterants are clove leaf oil and synthetic cinnamic aldehyde.

Cinnamon leaf oil is distilled from the leaves of *Cinnamomum zeylanicum*. It closely resembles clove oil, containing from 70 to 90% of eugenol. Small quantities of cinnamic aldehyde and terpenes are present.

The pure oil has a sp. gr. 1.044 to 1.065, optical rotation −0° 15′ to + 2° 20′; ref. index 1.5310 to 1.5400, and eugenol value from 70 to 90%.

Celery Oil.—All parts of the herb *Apium graveolens*, the common celery, yield an essential oil, of which that from the seeds is most valued. Schimmel

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[1] *Chem. and Druggist,* 1910, 76, 959.
[2] *Perf. and Ess. Oil Record,* 1910, 1, 169.

& Co., state that from the green leaves most exactly reproduces the natural celery flavour. The oil from the seed is obtained to the extent of about 3%, as a liquid of strong celery odour, having a sp. gr. 0.870 to 0.895 and an optical rotation +65° to +80°. The chief constituent of the oil is dextro-limonene. Ciamician and Silber[1] examined the high boiling fractions of the oil and found traces of palmitic acid, guaiacol and a crystalline substance of the formula $C_{16}H_{20}O_3$, m. p. 66° to 67°. In addition, a sesquiterpene was found, and 2 bodies, both acids, of a peculiar constitution, *sedanolic* and *sedanonic acids*, together with the lactone of the former, *sedanolide*, which appears to be the chief odorous constituent of the oil. *Sedanolic acid* $C_{12}H_{20}O_3$ a crystalline substance, m. p. 88° to 89°, is easily converted into its lactone, sedanolide $C_{12}H_{18}O_2$. *Sedanonic acid*, $C_{12}H_{18}O_3$ melts at 113°, and possibly occurs as an anhydride in the oil. These 2 acids are nearly related, and Ciamician and Silber consider that sedanolic acid is *o*-oxyamyl-tetrahydrobenzoic acid, and that sedanonic acid is a related ketonic acid.

Schimmel & Co.[2] isolated and described the sesquiterpene present in this oil, which they prepared by regenerating the hydrocarbon from its crude hydrochloride. Its characters were given as follows: b. p. 268–272°, sp. gr. 0.9196 at 20°, optical rotation +49° 30' and ref. index 1.5048. Semmler and Risse[3] consider that a second sesquiterpene has been present, and that after the elimination of this pure selinene should have a rotation of +61° 36' and a ref. index 1.5092.

Champaca Oil.

This oil, somewhat resembling ylang-ylang oil, is one of very high perfume value. It is distilled from the flowers of *Michelia Champaca*, a plant cultivated, and also growing wild, in the forests of the Himalayas, from Nepal and Kumaon eastwards; and also in the Nilghiris and Travancore, Java and the Philippines. The flowers, which are of an exquisite odour, are not unlike a double narcissus. The essential oil distilled from the flowers is known in India as *Pand* or *Champa-ka-utter*. The oil is known as *Yellow Champaca*, whilst that from *Michelia longifolia* is known as *White Champaca*. A sample of the former distilled in Java (yellow champaca oil) was a pale yellow oil of thin consistency, resembling, in a degree, oil of orris in odour. It had a sp. gr. 0.914, and an optical rotation −13° 14'. The oil of white champaca from the same source had a sp. gr. 0.883 to 0.897, an optical rotation −12° 50' and ref. index 1.4470. Its odour recalled that of basil. According to Schimmel & Co., the sp. gr. of the oil from *Michelia champaca* varies from 0.907 to 0.940 and the optical rotation from −12° 18' to −55°.

According to Bacon[4] the oil deposits crystals, and on standing for a time

[1] *Ber.* 1897, 30, 492.
[2] *Report,* April, 1910, 32.
[3] *Ber.,* 1912, 45, 3301.
[4] *Philippine J. Sci.,* 1910, 5, 262.

becomes semi-solid. Brooks[1] states that the oil has a sp. gr. of 0.904 to
to 0.9107 at 30°/30°, ref. index 1.4640 to 1.4688, and ester value after
acetylation 199. Benzyl alcohol and benzaldehyde are constituents of the
oil.

The leaf oil which is prepared on a commercial scale in Java has a sp. gr.
0.922, optical rotation $+12°$ 30', ester value about 25, and ester value after
acetylation about 60 to 65.

Clove Oil.

Clove oil has a very high ref. index, rarely falling below 1.5310 and usu-
ally reaching 1.5340.

Castor oil has recently been found as an adulterant of clove oil. This
adulterant lowers the sp. gr. and the ref. index, and causes the oil, although
soluble in 1.5 to 2 volumes of 70% alcohol, to become turbid on the addition
of more alcohol.

From the highest boiling fractions of oil of clove stems (b. p. 143 to 155°
at 9 mm.; α_D — 21°; $d^{20°}$ 0.966; $n_D^{20°}$ 1.5010) F. W. Semmler and E. W.
Mayer[2] have isolated a sesquiterpene alcohol, $C_{15}H_{26}O$, which they found to
possess the following constants: b. p. 138 to 148° (8 mm.), $d^{20°}$ 0.9681, α_D — 17°,
n_D 1.5010, mol. refr. found 68.18, calc. for $C_{15}H_{26}O/$ 68.07. Judging from
these values the substance is a bicyclic sesquiterpene alcohol with one double
bond. The chloride of the alcohol (b. p. 147 to 155° at 12 mm.; $d^{20°}$ 0.990)
when treated with alcoholic potash solution yields a hydrocarbon having
the following properties: b. p. 123 to 126° (10 mm.), $d^{20°}$ 0.9273, $\alpha_D^{20°}$ — 23°,
$n_D^{20°}$ 1.5024.

Clove leaf oil closely resembles clove bud oil in characters, and contains
from 78 to 88% of eugenol.

Oil of Cubebs.

The ref. index of oil of cubebs varies from 1.4935 to 1.4970.

Some spurious cubeb oil, distilled from a species of *Piper* not yet identified,
has recently been found on the market. Umney and Potter[3] found it to have
an optical rotation of about —14°.

In the fractional distillation of the oil, a considerable amount should pass
over between 250° and 280°. A sample having a sp. gr. 0.913, optical rota-
tion —24° and ref. index 1.4915 gave the following results on fractionation:

Below	175°..	Nil.
	180°..	4 %
	185°..	7 %
	190°..	10 %
	200°..	14 %
	210°..	18 %
	220°..	22 %
	230°..	25 %
	240°..	28 %
	250°..	33 %
Above	250°..	67 %

[1] *Philippine J. Sci.*, 1911, 6, 333.
[2] *Ber.*, 1912, 45, 1392.
[3] *Chem. and Druggist*, 1912, 80, 331, 443.

The following results were obtained on cubeb oils, recently distilled by Messrs. Stafford Allen & Sons, Ltd.

Sp. gr. at 25° C.	Rotation 100 m /m.	Ref. index at 25° C.	Distillate approx. %	
			Under 250°	250°/280°
0.919	−29.4	1.4928	21.6	75.0
0.920	−29.4	1.4930	29.0	66.0
0.923	−29.1	1.4950	17.0	78.0
0.924	−28.5	1.4944	32.0	64.0
0.923	−27.2	1.4944	14.0	80.0
0.924	−27.4	1.4949	22.0	72.0
0.927	−27.2	1.4950	50.0	44.0

Cypress Leaf Oil.

The oil from the leaves of *Cupressus sempervirens* has during the past few years become very popular as a remedy for whooping-cough.

The oil contains a mixture of terpenes, a ketone resembling thujone, a sesquiterpene, cedrol, sabinol, a sesquiterpene alcohol, and esters of terpineol.

The oil has the following characters:

	German distilled	French distilled	Algerian distilled
Sp. gr.	0.880–0.900	0.868–0.884	0.8764
Rotation	+4° to +18°	+12° to +31°	+22° 18′
Ref. index	1.4740–1.4800	1.4710–1.4760	....
Acid value	1.5 to 3.0	0 to 2	....
Ester value	13 to 22	3 to 14	....
Ester value after acetylation	36 to 57	9 to 32	....

Eucalyptus Oil.

Numerous new species of eucalyptus oil have been described, but as they add nothing to the commercial and little to the chemical knowledge of this oil they need no detailed descriptions.

Estimation of Cineol.—The resorcinol method, advocated by Schimmel & Co., has not met with universal approval (Vol. IV, p. 341). In cases where the oil is very rich in cineol, the contents of the flask set to a solid mass and no reading is possible. This is to some extent obviated by diluting the oil with an equal volume of petroleum, and making the necessary correction in reading the unabsorbed portion. There is also no doubt[1] that other oxygenated constituents than cineol are absorbed, and that the process is not very accurate.

Dodge,[2] in a communication to the 8th Inter. Con. of Appl. Chem. has suggested another method for the estimation of cineol.

[1] Bennett, *Chem. and Druggist*, 1908, 1, 55; *Perf. and Ess. Oil Record*, 1912, 269.
[2] *J. Ind. Eng. Chem.*, 1912, 4, 259

This is based upon the familiar fact that, in the cold, cineol remains practically unattacked by potassium permanganate, whereas the remaining constituents of the oils in question (eucalyptus oil and cajuput oil) are oxidised into soluble compounds. The process is carried out as follows: 10 c.c. of the oil are placed in a narrow-necked flask, cooled with ice-water, and shaken with a gradually-added 5 to 6% solution of potassium permanganate, until the latter is present in excess. The mixture is then left standing in ice-water for from 12 to 18 hours with occasional shaking, after which the manganese peroxide which has separated is brought into solution by carefully adding sulphurous acid (or sodium hydrogen sulphite + hydrochloric acid). The unattacked oil (eucalyptol) is brought into the neck of the flask, pipetted into a graduated tube, washed with a little alkali, and estimated volumetrically. Its sp. gr. should be 0.929 to 0.930 (15°); it should be inactive, and dissolve in 3 to 5 volumes of 60% alcohol at 25°.

Bennett[1] has carried out a series of estimations with the following results:

	Cineol by permanganate process.	Cineol by phosphoric acid process	Resorcinol process
	%	%	%
Eucalyptol.........................	98	....	....
Oil of eucalyptus, b. p................	84	70	89
Oil of eucalyptus globulus............	80	68	65
Oil of eucalyptus amygdalina.........	78	....	....
Oil of cajuput, good quality..........	80	52	54
Oil of cajuput, fractionated..........	70	14	17

It is evident from these experiments that, although the process may give approximate results with eucalyptol itself and with eucalyptus oils of high cineol content, it is not to be relied upon for cajuput oils or for eucalyptus oils of the amygdalina type, since these oils evidently contain constituents which are not readily oxidised by cold solution of permanganate. Further experiments are being made in order to determine to what extent terpenes and sesquiterpenes are oxidised under different conditions. The process is easily carried out, and, if it can be modified to give accurate results, it would prove a valuable addition to analytical methods for essential oils.

Fennel Oil.

This oil is distilled from the fruit of several varieties of *Fœniculum vulgare*, which is found all over Europe except in the north and northeast, being especially common on the Mediterranean littoral; it is also found in Asia Minor, Persia, India and Japan. Two oils are recognised in commerce, the "sweet" and "bitter" oils, the former being more esteemed. The sweet fennel is said to be *Fœniculum sativum*, but this is probably only a variety of the common wild fennel. The great variability in the fruits of different

[1] *Perf. and Ess. Oil Record*, 1912, 295.

districts makes it necessary to fix some limits for the physical characters of the oil, which, however, may be exceeded in individual cases. The terpenes, pinene, phellandrene, dipentene and limonene, have all been detected in fennel oils, and the ketone fenchone, and anethole. All these bodies, however, may not occur in any given sample. Upon the presence of anethole the value of the oil chiefly depends and the solidifying point of the oil is therefore a fair criterion of its value, if the oil is pure. A good oil will, according to Parry, contain as much as 60% of anethole. The sp. gr. should not fall below 0.960 nor above 0.980, and the optical rotation should vary from $+6°$ to $+20°$. The solidifying point (see *Oil of Aniseed*) should not fall below $+5°$. If necessary the crystalline stearoptene may be separated and examined, but as a rule added solid bodies will alter the other characters of the oil. The above tests will guard against the abstraction of anethole, or the addition of the residue of oil from which this body has been abstracted.

Geranium Oil.

The following are now accepted as the characters of the various types of geranium oil.

	Sp. gr.	Rotation	Ref. index	Esters
Réunion oil	0.888–0.896	-7 to $-14°$	1.462–1.468	21–33%
African oil	0.892–0.904	$-6°$ 30' to $-12°$	1.465–1.472	15–30%
French oil	0.896–0.905	$-7°$ to $-11°$	1.463–1.469	19–28%
Spanish oil	0.897–0.907	$-7°$ to $-11°$	1.462–1.469	27–42%
Corsican oil	0.896–0.901	$-8°$ to $-11°$	1.461–1.471	22–27%
German oil	0.906	$-16°$	....	28%

By using the acetylation and formylation processes, the following results have been obtained by various observers, in regard to the percentage of total alcohols and of citronellol present in different types of geranium oil:

Oil	Total alcohols	Citronellol	Observer
African	69–79%	32–43%	Simmons
Réunion	69–73%	44–51%	Simmons
Corsican	69.8%	30.3%	Simmons
Trappe de Staouéli	71.5%	27.9%	Simmons
French	72.7%	39.8%	Umney
Algerian	74.1%	32.9%	Umney
Bourbon	73.0%	44.3%	Umney
Corsican	73.7%	45.9%	Umney
Asian	69–72%	51–62%	Umney

Artificial esters are now a common adulterant of geranium oil. Details as to the detection of these are to be found on pages 330 to 336.

Parry[1] has detected ethyl oxalate as an adulterant of this oil. He gives the following particulars in reference to this adulteration.

Ethyl oxalate is a colourless, somewhat aromatic, oil of sp. gr. 1.079 at

[1] *Perf. and Ess. Oil Record*, 1911, 83.

20°, and b. p. 186°. It appears as rather more than twice its weight of geranyl acetate, or still more of geranyl tiglate.

The oils in question had the following characters:

	Sp. gr. at 15°	Ref. index at 20°	Apparent ester value, %	Rotation
1.	0.9197	1.4703	57.5	−10° 40′
2.	0.9093	1.4702	46.0	−10° 35′
3.	0.9225	1.4634	74.0	−10° 45′

Within a few minutes of the commencement of saponification under a reflux condenser, a good crop of silky crystals appears in the flask, due to the formation of potassium oxalate which crystallises out. The saponification liquid, freed from alcohol, and filtered from the decomposed oil, contained oxalic acid which was identified by the usual tests.

In an exhaustive classification of the geranium species and their odours, the reader is referred to an elaborate article on the genus pelargonium by E. M. Holmes.[1]

Ginger Oil.

This oil is distilled from the rhizomes of *Zingiber officinale*, a native of tropical Asia, which is cultivated in the East and West Indies, Africa, and to a small extent in Australia.

The constituents of the oil are as follows: *d*-camphene, β-phellandrene, cineol, citral, borneol, geraniol (?), the sesquiterpene zingiberene, and decylic aldehyde. Ginger oil has the following characters:

Sp. gr.	0.875 to 0.886
Optical rotation	−28° to −50°
Acid value	0 to 2
Ester value	0 to 15
Ester value after acetylation	33 to 42
Ref. index	1.4795 to 1.4855

Ginger oil is very sparingly soluble in alcohol, requiring from 6 to 10 volumes of 95% alcohol to give even an opalescent solution. According to Thresh, the English distilled oil yields the following fractions on distillation:

	%
Below 150°	5
150°–200°	10
200°–240°	8
240°–265°	60
265°–300°	7
Residue	10

These results yield but little information, however, as decomposition goes on to a slight extent during distillation at ordinary pressures.

Hop Oil.

This oil is distilled from the flowers of *Humulus lupulus*. It is an oil of pronounced odour of hops, and contains the following constituents: myrcene, linalol, an acid which exists in the form of esters, and which is probably

[1] *Perf. and Ess. Oil Record.* 1913. 239.

isononylic acid; geraniol, α-caryophyllene, and β-caryophyllene (the two last named appear to be the true constituents of the body named *humulene* by Chapman (*Trans.*, 1895, **67**, 54, 780). Genuine hop oil has the following characters:

Sp. gr.	0.855 to 0.895
Optical rotation	−1° to +1°
Ref. index	1.4850 to 1.4925
Acid value	0 to 10
Ester value	15 to 40
Ester value after acetylation	about 70 to 80.

Juniper Berry Oil.

This oil (*vide* page 345, Vol. IV) is sometimes slightly dextrorotatory (Russian oil) and is also as highly lævorotatory as −19° (Hungarian oil).

The constituents of juniper berry oil are α-pinene, camphene, terpinenol, geraniol (?), borneol (?) and cadinene. Terpineol does not appear to be present. In distilling juniper oil fractionally, the last 20% should have a ref. index of 1.4950 to 1.5120. Adulteration with much turpentine will cause this figure to be lowered.

It is to be remembered that juniper oil alters in character to a very considerable extent by keeping. Its sp. gr. rises and its solubility decreases, so that old juniper oil will not fulfill the requirements of the various Pharmacopœias.

Lavender Oils.

The following bodies have recently been discovered in lavender oil (from *Lavendula vera*). Elze[1] has isolated the alcohol nerol, and the phenol, thymol; and Schimmel & Co.[2] have found the sesquiterpene, caryophyllene.

Improvements in the methods of distillation appear to be responsible for occasional increases in the ester values of French and the other foreign lavender oils. A sample distilled at Barrême[3] was found to contain 55.7% of esters, which is confirmed by Schimmel & Co.[4] who have found as much as 56% for oil distilled in this district. Samples distilled in Dalmatia have also been found to contain from 43 to 57%.[5]

The sp. gr. of genuine lavender oils will sometimes fall to 0.880, but in such cases care should be exercised in judging all the other analytical figures of such an abnormal oil. Old oils will often be found with abnormally high sp. gr., so that the age of the oil must be taken into account. For the tendency of true and spike lavender flowers to hybridise, see Birckenstock.[6] The hybrid plants are known as "spigouse" and "lavandin," and the oils therefrom are midway in characters between lavender and spike oils.

Two typical lavandin oils gave the following results on analysis:

[1] *Chem. Zeit.*, 1910, **34**, 1029.
[2] *Report*, April, 1913, 70.
[3] *Perf. and Ess. Oil Record*, 1913, **4**, 134.
[4] *Report*, October, 1913, 68.
[5] *Perf. and Ess. Oil Record*, 1913, **4**, 153.
[6] *Schimmel's Report*, Oct., 1906.

	1	2
Sp. gr...........................	0.9027	0.8995
Optical rotation...................	−0° 43′	−1° 35′
Ester value.......................	6.23%	9.12%
Total alcohols....................	34.8%	36.5%

For the adulteration of lavender oil with artificial esters, see under "Esters" (page 330).

The following table[1] shows the influence of certain adulterants on lavender oil. Samples Nos. 1 and 2 are adulterated with terpinyl acetate. No. 3 was abnormal, but as shown by an examination of the oil after steam distillation, the abnormalities were due to resinification, rather than to adulteration. No. 4 was adulterated with glyceryl acetate, and No. 5 is a pure high ester oil which gave identical, or nearly identical results, when different quantities of potash were used for saponification.

Lavender oil	No. 1	2	3		4		5	
			Original oil	Dist. with steam	Original oil	Shaken once with 5% alc.	15 c.c. seminormal	10 c.c. potash solution
d¹⁵	0.9007	0.8946	0.9072	0.8932	0.8992	....	0.8913	......
αᴅ.....................	−3° 55′	−2° 34′	−5° 14′	−5° 37′	−4° 32′	....	−8° 43′	......
Solub. in 70% alcohol.....	2.5 vol.	2.5 vol.	1.9 vol.	2.2 vol.	2.5 vol.	....	4.5 vol.	......
Acid value..............	0.3	0.5	0.8	....	0.5	....	0.3	0.3
Ester value after 1 h. sap. with 10 c.c. N/2 solution.	78.4	83.3	100.5	102.5	90.0	87.0	145.7	143.4
Ester content (calc. for linalyl ac.)..............	27.4%	29.2%	35.2%	35.9%	31.5%	30.4%	51%	50.2%
Ester value after 2 h. sap. with 20 c.c. ¼ N. solution	82.7	87.6	104.2	104.6	91.9	....	146.0	146.0
Ester value after 1 h. sap. with 10 c.c. ½ N. solution +25 c.c. alc	69.7	77.0	99.0	100.6	91.1	....	144.1	132.9
Difference................	13.0	10.6	5.2	4.0	0.8	....	1.9	13.1
Sap. value...............	78.7	83.8	101.3	102.5	90.5			
Acid value II............	75.7	83.7	96.7	100.6	90.6			
Difference................	3.0	0.1	4.6	1.9	....			
Terpinyl acet. content about	5%	4%	} doubt- ful		not ascertainable			
Esters of spar. vol. acids...	not ascertainable				not ascertainable			
Glyceryl ester...........	not ascertainable				not asc.	present		

In regard to Spanish spike lavender oil, it is to be noted that the optical rotation of the oil (as well as of the first 10% distilled) is more often than not to the left.

Commercial spike oils, especially Spanish oil, are almost bound to contain small quantities of labiate oils other than true spike oil, because it is quite impossible to separate the flowers which are to be found growing side by side with genuine *Lavandula spica*, so that a mixture of these with a small quantity of allied species is usually thrown into the still. The following are several varieties of lavender which are occasionally distilled.

The oil from *Lavandula pedunculata* has a sp. gr. 0.939 and optical rotation −45°. It contains nearly 40% of esters, in addition to cineol and pos-

[1] *Schimmel & Co., Report,* April, 1912, 86.

sibly thujone. The oil of *Lavandula Stæchas* (the "holy rosemary" of the Spaniards) resembles rosemary rather than lavender in its odour. Its sp. gr. is about 0.940. The oil of *Lavandula dentata*, of sp. gr. 0.926, also resembles rosemary, with a marked camphoraceous odour. Charabot[1] records the examination of a sample of Spanish lavender oil, but does not state its source. He found in it much free linalol, but only 3% of esters. Borneol was also present.

Lemon Oil.

A favourite adulterant of lemon oil is a highly purified Greek turpentine, fortified with lemongrass citral. Greek turpentine is obtained from *Pinus halapensis*, and has the following characters:

```
Sp. gr. .....................................  0.861  to 0.486
Optical rotation. ............................  +33° to +39°
Ref. index. .................................  1.4675 to 1.4705
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The analytical figures for lemon oil vary considerably at different periods of the pressing, and care should be taken to examine, as far as possible, authentic samples from various districts, pressed at different times, in order to assist one in discriminating between adulterated and merely low-grade samples.

The ref. index of pure lemon oil is usually 1.4750, rarely below 1.4750 and in the writer's experience never below 1.4740.

In certain seasons the sp. gr. may rarely fall to 0.855, and where only spring lemons are used even to 0.854, and the optical rotation will sometimes be found as low as +55.

All pure lemon oil contains a small quantity of pinene, and the writer considers that Chace's method of detecting turpentine in lemon oil (Vol. IV, p. 356) must be used with considerable caution.

Lime Oil.

This oil is obtained either by an expression process (generally by the ecuelle) or by distillation, from *Citrus limetta* (Italian) or *Citrus medica var. acida* (West Indian). The pressed oil is the superior of the two and commands a much higher price. Italian lime oil, which is expressed, not distilled, is of a brownish-yellow colour and has a characteristic fragrant odour of the fruit with a secondary odour of bergamot. Its sp. gr. varies from 0.870 to 0.875, and its rotation from +34° to +40°. It contains linalyl acetate and citral. It also contains a little free linalol, but not more than 3 or 4%. The bulk of the oil consists of the terpene limonene.

The West Indian oil, which is the usual oil of commerce, is obtained from the fruits of *Citrus medica var. acida*, whose juice contains a large quantity of citric acid. The plant is plentiful in Jamaica, Dominica and Tahiti; but the most important plantations are on the island of Montserrat, one of the Antilles. The lime harvest here lasts from September to January, and the chief product is, of course, the lime juice.

[1] *Bull. Soc. Chim.*, **17**, 378.

It is well known that distilled lime oil is quite different from the hand-expressed oil, the first-named oil having a disagreeable, turpentine-like odour. H. A. Tempany and N. Greenhaigh,[1] who have investigated the matter are of opinion that the difference is caused by the loss, during the process of distillation, of part of the lowest and highest boiling fractions. They distilled hand-expressed oils with steam, and obtained an oil with the characteristic turpentine-like odour of the distilled oil of commerce. The highest boiling fractions of the hand-expressed oil contain a blue fluorescent, crystalline body (perhaps methyl anthranilate), which is absent from the distilled oil. Moreover, limettin, which ordinarily separates out from the hand-expressed oil when it is left standing, is absent from the distilled oil. As a rule the citral-content of the distilled oil is lower than that of the hand-expressed oil. The authors found authentic samples to show the following properties:

I. Hand-expressed oils: $d^{30°}$ 0.8712 to 0.8859, $\alpha_D^{31°}$ + 31.38 to 33.43°, $n_D^{32°}$ 1.4789 to 1.4851, acid value 1.35 to 2.8, citral content 2.2 to 6.6%.

II. Distilled oils: $d^{30°}$ 0.8540 to 0.8858, $\alpha_D^{31°}$ +33.09 to 34.89°, $n_D^{32°}$ 1.4702 to 1.4713, acid value 0.76 to 1.3 citral content 1.2 to 2.0%.

Linaloe Oil.

The linaloe oil of ordinary commerce is distilled both in Mexico and Europe from the wood of several species of *Bursera*, chiefly from *Bursera Delpechiana* and *Bursera aloexylon*, and is in no way connected with aloe wood, as its name implies (*lignaloe*). A linaloe wood is also exported from French Guiana and Brazil, but this, known locally as *bois de rose femelle* or *licari* wood, is the product of one of the *Lauraceœ*, probably *Ocotea caudata*. Its essential oil is known as cayenne linaloe oil, or oil of Bois de Rose.

The principal odorous substance of both oils is the alcohol, linalol. In addition Mexican linaloe oil has been found to contain geraniol, linalol oxide, methyl-heptenone, a sesquiterpene, two terpenes, methyl-heptenol, terpineol, and myrcene. Cayenne linaloe oil contains also terpineol, geraniol, methyl-heptenone, methyl-heptenol, nerol, cineol, dipentene and probably myrcene.

The general characters of the two oils usually fall within the following limits:

	Mexican	Cayenne
Sp. gr.	0.870–0.880	0.875 to 0.898
Rotation	−10° to −19°	+8° to −12°
Ref. index	1.4610–1.4630	1.4590–1.4645
Acid value	1–3	1–2
Ester value	5–9	3–20 (rarely 40–75)
Free alcohols	85–96%	84–94%
(Determined in xylene solution).		

[1] *West Indian Bull.*, 1912, 12, 498.

Both oils are soluble in 2 to 3 volumes of 70% alcohol. It must be remembered that the estimation of linalol must be carried out with the modifications in the acetylation process described under the estimation of free alcohols, xylene being the best diluent.

The behaviour of the oil on fractional distillation affords a useful indication of the purity and quality of the sample, a high linalol value being indicated by a close similarity in the various fractions constituting the first 90% distilling. A typical sample distilled by the writer and Bennett, having a sp. gr. 0.882 and an optical rotation −11°, gave the following results:

Fraction	%	Sp. gr.	Rotation	Ref. index
1	10	0.808	−11°	1.4580
2	20	0.870	−11°30′	1.4590
3	20	0.871	−13°	1.4605
4	20	0.872	−13°	1.4612
5	20	0.876	−120°	1.4620
6	10	0.913	....	1.4750

An oil known as shiu oil distilled in Formosa from a species of cinnamon (?) contains a large amount of linalol, and may be expected to become an adulterant of linaloe oil, which is largely used in perfumery, and for the manufacture of artificial perfumes.

Shiu oil, however, contains camphor, and as it is very difficult to remove the whole of this, the presence of camphor in linaloe oil may be regarded as indicative of the presence of shiu oil. Schimmel & Co.[1] give the following methods for its detection.

Detection of Camphor by Means of Semi-carbazide Hydrochloride.—25 grm. of oil, distilled by steam, are diluted with a mixture of 1 grm. of semi-carbazide hydrochloride and 1 grm. of sodium acetate in 25 c.c. of 90% alcohol. After standing for 24 hours, 1 grm. is added and the whole mass distilled by steam. The distillation-residue is slightly evaporated in a dish on the water-bath and cooled. This causes the semi-carbazone to separate out from the camphor. A little hydrazodicarbonamide (decomposition product of the semi-carbazide hydrochloride) may be found admixed with the latter. The semi-carbazone is now filtered off and dissolved in a little alcohol, the hydrazodicarbonamide remaining undissolved. When the alcohol has evaporated the camphor semi-carbazone is left; when decomposed with dilute sulphuric acid the latter should develop a clearly perceptible odour of camphor. As a rule the semi-carbazone does not melt very sharply, because the substance is still contaminated with certain other compounds which can only be removed by repeated recrystallisation, but the quantity of the substance available is often insufficient for the purpose.

So small an addition to linaloe oil as 1% of camphor can be traced by the semi-carbazide method, especially when the mixture is allowed to stand for

[1] *Report*, Oct., 1913, 71.

more than 24 hours. But the method is not suitable for quantitative esti-
mation; for example, after one single treatment of a mixture of 80% linalol
and 20% camphor only 11% camphor was traceable after an interval of 2
days. The method has, moreover, the great drawback of being very slow.

Estimation of Camphor by Oxidation with Potassium Permanganate.—
Much better results were obtained by this method, which made it possible to
estimate the camphor-content rapidly and with great accuracy.

The *modus operandi* is as follows: In a distilling flask of 2 litres capacity,
50 grm. of potassium permanganate and 300 c.c. of water are placed; within
the course of 30 minutes, 10 grm. linaloe oil are added gradually through a
dropping funnel, cooling strongly with ice. The oil is dissolved by warming
it. When the action is quite completed and no further warming takes place
(after about 2 hours) a further 200 c.c. of water are added and the unattacked
camphor is driven over with steam.

By this method it was possible to detect the addition to linaloe oil of 1%
camphor and 10% shiu oil. The manipulation can be carried out in about
4 hours, and the method is also suitable for the quantitative estimation of
camphor in linaloe oils which have been adulterated with shiu oils, for which
purpose it is necessary to shake out the steam-distillate several times with
ether after adding common salt. The ethereal extracts are placed together
and the residue is weighed after evaporating the ether.

Orange Oil.

The presence of citral and citronellal in orange oil is doubtful, indeed,
highly improbable.

The statement that oil of orange has a higher optical rotation than any
other essential oil (see Vol. IV, p. 360) is inaccurate.

Jamaican orange oil is now a regular commercial article, and although
it differs slightly from Sicilian oil, the two products are very nearly identical.
The writer has examined samples of this Jamaican oil with the following
results. A sample of sweet oil had the following characters:

Sp. gr. at 15°.. 0.850
Optical rotation..+98°40
Ref. index at 20°.. 1.4719

On fractionation under 12 mm. pressure, the oil gave the following frac-
tions:

10 c.c. of rotation + 97° 30′ and ref. index 1.4709
80 c.c. of rotation + 99° 20′ and ref. index 1.4707
5.7 c.c. of rotation + 86° 53′ and ref. index 1.4705

The total aldehydes present were found to be 1.2%.

Two other samples of West Indian orange oil which were examined under
similar circumstances contained 1.3 and 1.4% of aldehydes.

Compared with Sicilian oils the above figures show no appreciable

difference, the figures being practically identical, and the aldehyde value of such oils averaging 1.3%.

Oil of Rue.

The herb, *Ruta graveolens*, yields an essential oil characterised by its extremely low sp. gr. The oil is of a pale yellow colour and characteristic odour, and consists almost entirely (at least 90%) of methyl-nonyl-ketone, $CH_3.CO.C_9H_{19}$, with a small amount of methyl-heptyl-ketone. The sp. gr. of the oil varies from 0.830 to 0.847, and it is usually slightly dextrorotary, from 0° to +2° 30'. When exposed to the action of a freezing mixture the oil solidifies to a crystalline mass at +9° to +10°. It begins to boil at 215°, and is completely distilled at 232°. It should dissolve to a clear solution with from 2 to 3 volumes of 70% alcohol. Pure methyl-nonyl-ketone is a bluish oil, m. p. +15° and b. p. 225°. The fluorescent substance present is the methyl ester of methyl-anthranilic acid. The characters given below are sufficient to guard against adulteration, which, however, is frequently practised, the usual adulterant being turpentine and, more rarely, petroleum, which, however, alter the constants of the oil so much as to be very easily detected. Oil of rue is not largely employed in medicine, and has occasionally been employed for illegitimate purposes.

The less important constituents of this oil are pinene, limonene, cineol, and, in Algerian oil, traces of methyl-salicylate. The following are the characters of the principal varieties of the oil.

	French	Algerian	Spanish
Sp. gr.	0.830–0.845	0.837–0.845	0.833 to 0.848
Rotation	−0° 40' to +2° 10'	0° to +1°	−1° to +1°
Ref. index	1.430–1.434	1.430–1.433	1.431–1.434
Solidifying point	5° to 10°	7° to 11°	−2° to +8°

Petitgrain Oil.

A number of parcels of petitgrain oil have been found during the last few years, having a somewhat low ester value, and a high optical rotation. This is probably due to the more or less careless selections of the material to be distilled, in which some young fruits, more mature than usual, have been included. The ester value of such oils falls to 35%, and the optical rotation rises to +9°. A sample of petitgrain oil, of authentic origin, distilled in Jamaica[1] has been found to have a rotation −6° 45'.

Terpinyl acetate has recently been found as an adulterant of this oil. The following results are obtained on the analysis of several samples of petitgrain oil so adulterated. The analyses are by the writer and by Messrs. Schimmel & Co.

[1] *Bull. Imp. Instit.*, 1913, 11, 437.

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	1	2	3	4
d^{15}	0.9019	0.8968	0.8917	0.8905
α_D	+4° 47'	+1° 40'	+2° 56'	−12° 0'
Solubility	5 vol. & m. 70 p.c. alc. dilute sol. opal.	3 vol. & m. 70 p.c. alc.	1 vol. & m. 80 p.c. alc. dilute sol. opal.	Any vol. 90 p.c. alc.
Acid value	2.3	0.3	0.5	0.5
Ester value after 1 h. sap. with 10 c.c. N/2 alkali	111.2	112.4	136.8	84.0
Ester content (calc. as linalyl acetate)	38.9 p. c.	39.4 p. c.	47.9 p. c.	29.4 p. c.
Ester value after 2 h. sap. with 20 c.c. semi-normal alkali	119.3	121.8	138.5	89.0
Ester value after 1 h. sap. with 10 c.c. semi-normal alkali + 25 c.c. alc.	102.3	99.7	133.5	80.5
Difference	17.0	22.1	5.0	8.5
Sap. value	113.5	112.7	137.3	....
Acid value II	107.6	112.4	128.4	....
Difference	5.9	0.3	8.9	....
Terpinyl acetate content about	9.p.c.	12.p.c.	....	....

Peppermint Oil.

Japanese peppermint oil has been found to contain Δ'-menthenone, a ketone not hitherto found in essential oils.[1] Lævo-limonene, which Power isolated from American oil has been found by Murayama[2] in Japanese oil. Messrs. Schimmel & Co.[3] have identified d-ethyl-n-amylcarbinol as a constituent of the Japanese oil. Neo-menthol is also present in this oil.[4] Thoms has carried out a series of experiments on the cultivation of the Japanese plant at Dahlem near Berlin and in German South West Africa. The characters of the oils obtained are compared in the following table with those of normal Japanese distilled oil.

	Old Jap. oil (dist. in Japan)	Oil from herb grown in German S. W. Africa	Oil from herb grown at Dahlem (summer 1911)
Sp. gr.	0.9043 (18°)	0.9032 (22°)	0.8954 (22°)
α_D	−35.25° (23°)	−35° (24°)	−34.75° (25.5°)
Acid value	4.05	2.99	4.01
Ester value	27.73	4.68	12.744
Ester value after acet.	289.5	304.8	283.25
Esterified menthol	7.74	1.302 %	3.546
Free menthol	72.77	83.528 %	75.271
Total menthol	80.51	84.830 %	78.817
Sol. p.	cannot be determined	+20 to 20.5°	+14.5°

Conversely, Shinosaki[5] has examined oils distilled from German and English plants grown in Japan. The following are the figures which these oils gave on analysis.

1 *Schimmel's Report*, Oct., 1910, 97.
2 *J. Pharm. Chim.*, 1910, vii, 1, 549.
3 *Report*, April, 1912, 103.
4 Pickard and Littlebury, *Trans.*, 1912, 101, 109.
5 *J. Ind. Eng. Chem.*, 1913, 5, 656.

Oil from	Japanese herb	German herb		English herb		German herb from Okayama
		1910	1911	1910	1911	
$d^{15°}$	0.8989	0.9638 (?)	0.9105	0.9228	0.9132	0.9161
a_D	−28.92°	....	−42.25°	−52.25°	−63.60°	−18.15°
n_D^{20}	1.4602	1.4671	1.4672	1.4717	1.4573	....
Acid value	0	19.53	0	4.42	7.06	3.45
Menthyl acetate	6.35	24.94	11.08	26.50	13.65	8.36
Total menthol	69.30	85.71	66.30	66.88	75.60	58.61
Sol. in 70% alcohol at 20° } in vols.	2.8	2.5	insol. 15	insol. 15.3		insol.

In regard to the botanical origin of the Japanese peppermint plant, differences of opinion exist. For details of these, a paper by Holmes[1] should be consulted.

The following details in regard to French oil of peppermint have been given by Camus.[2]

Mentha piperita does not constitute a separate species, but is a hybrid of *Mentha viridis* and *Mentha aquatica*. It may be regarded as a sterile plant, as it fruits rarely, and even then the fruit is mostly badly developed, hence the plant must be propagated by subdividing the rhizomes. Like all *Mentha* species, *Mentha piperita* is extraordinarily variable. Too add to the confusion, different varieties are cultivated in various plantations under the common denomination of "peppermint." Several varieties also occur of the original species. *Mentha piperita* embraces 2 groups; group I including the sub-species *piperita*, Briq. with numerous varieties, while group II includes the sub-species *citrata*, Briq., which under the name of "citronella" is much grown in France because of its pleasant aroma, but does not appear to be used commercially. In its internal structure *Mentha piperita* exhibits certain features midway between those of *Mentha viridis* and *Mentha aquatica*. How variable are the *Mentha*-species is shown by *Mentha viridis*, L. and *Mentha aquatica*, L.; for the authors describe no fewer than 6 varieties of the former, and as many as 8 of the latter, besides indicating many others.

The following figures are obtained from the ordinary oil, and the oil distilled from the so-called "red" peppermint, which has only recently been cultivated in France.

	Ordinary peppermint		Red peppermint	
$d^{15°}$	0.9191	0.9184	0.9170	0.9136
a_D	−10° 54′	−8° 3′	−16° 38′	−13° 44′
Solubility in 80% alcohol	1 vol. afterwards clouding	....	1 vol. afterwards clouding	....
Acid value	0.8	0.8	1.0	1.2
Ester value	40.5	31.7	18.9	17.5
Menthyl acetate	14.3%	11.2%	6.7%	6.2%
Ester value after acet.	160.8	169.1	180.2	185.6
Total menthol	50.9%	53.9%	58.0%	60.0%
Free menthol	39.6%	45.1%	52.8%	55.1%
Proportion of menthone	7.3%	....	16.8%	....

[1] *Perf. and Ess. Oil Record*, 1913, 4, 32.
[2] *Report of Roure-Bertrand Oils*, Oct., 1911, 3.

The limits given in Vol. IV, p. 373, for French peppermint oil are too narrow; the oil may have an optical rotation up to $-35°$, and an ester value equivalent to 27% of menthyl acetate. The same remark applies to Italian oils, which may have a rotation up to $-27°$.

The experimental station for medicinal plants attached to the Royal Hungarian Agricultural Academy at Klausenburg-Kolozsvár has examined a series of peppermint oils distilled in Hungary.

The Hungarian peppermint oil resembles the American oil closely. The plant yields 1.17% oil, possessing the following constants: $d^{15°}$ 0.90142 to 0.91918, α_D $-26.72°$ to $-32.38°$, $n_D^{20°}$ 1.4632 to 1.4760, soluble in 3 to 5 parts 70% alcohol and in its own volume of 90% alcohol. The following fractions passed over during distillation: up to 200° 7.0%, 200 to 205° 10.3%, 205 to 210° 13.4%, 210 to 215° 12.9%, 215 to 220° 16.7%, 220 to 225° 11.6%, 225 to 235° 16.4%.

Umney[1] gives the following colour reaction to distinguish Japanese from other peppermint oils:

1 c.c. of the oil is heated with 0.5 grm. of a mixture of equal parts paraformaldehyde and citric acid over a water-bath. With Japanese peppermint oil there is no colouration, whereas with American, English, Italian and German oils, a purple colour develops. This new test has the advantage of being quicker than the familiar colour test with concentrated acetic acid.

Spearmint Oil.

Nelson[2] has identified phellandrene as a constituent of this oil. Dihydrocumic acetate has been found in German spearmint oil by Elze,[3] and Nelson (loc. cit.) has found dihydrocarveol acetate in American oil.

Irk[4] has examined Hungarian spearmint oil which had the following characters:

Sp. gr. 15°/4°	= 0.9375 to 0.9513
Optical rotation	= $-44.4°$ to $-49.9°$
Ref. index	= 1.4899 to 1.4931
Carvone	62–71 %

It is soluble in its own volume of 80% alcohol.

Pine Needle Oil.

Böcker and Hahn[5] have isolated a new *aldehyde*, $C_{15}H_{20}O$, a new *ketone*, $C_{15}H_{24}O$, and a third compound, $C_8H_{14}O$, which has been named *pumilone*, from the oil of *Pinus pumilio*.

[1] *Perf. and Ess. Oil Record*, 1911, 2, 275.
[2] *U. S. Dept. Agric., Bur. Chem., Circular* No. 92.
[3] *Chem. Zeit.*, 1910, 34, 1175.
[4] *Pharm. Central.*, 1911, 52, 1111.
[5] *J. prakt. Chem.*, 1911, II, 83, 489.

Rose Oil.

The determination of the relative proportions of geraniol and citronellol in otto of rose is now recognised as one of the principal analytical factors in the examination of the oil. The amount of citronellol present varies with the country of origin of the sample. In Bulgarian otto of rose it is usually about 30–33%, and any sample falling below 28% should be regarded with considerable suspicion.

The adulteration with geraniol, if the adulterant be derived from palmarosa oil is sometimes indicated by the presence of gurjun balsam oil, which has been used as an adulterant of the palmarosa oil. Much gurjun oil is indicated by a high lævorotation, and samples with this value in excess of $-4°$ should be carefully examined. As little as 0.5 to 1% of gurjun oil may be detected by adding 5 or 6 drops of the sample to 10 c.c. of acetic anhydride containing 5 or 6 drops of nitric acid. A distinct purple colour develops if gurjun oil be present, the depth depending on the amount of the adulterant.

In the examination of otto of rose it must be remembered that the characters vary from season to season, and there is, in the writer's opinion, no essential oil more difficult to judge than this one. An expert nose will often yield as much or more information than the analytical values.

The following notes will afford some information in regard to ottos of rose other than Bulgarian, which are now to be found on the market.

Anatolian Otto.—This otto may have a high m. p. varying from 19° to 26°, and a sp. gr. up to 0.8635. The following represent typical samples, the figures varying according to the stearoptene content:

Sp. gr. 30°/15°	0.863	Sp. gr. 30°/15°	0.850
Optical rotation	$-2°\ 30'$	Optical rotation	$-3°$
Ref. index at 25°	1.4650	Ref. index at 25°	1.4600
Ref. index, after washing	1.4652	M. p.	23° to 24°
M. p.	21° to 22°	Citronellol	41.1%
Stearoptene content	15.8	Total alcohols	66.8%
Total alcohol, calculated as geraniol.	72.8%	Stearoptene	18.9%
Citronellol	36.2%		

French Otto.—This otto has recently become a commercial article. Very few authentic samples have been examined, but it appears to have characters which differ within wide limits, and it must, at present, be very largely judged by its odour. The sp. gr. of apparently genuine samples has been found up to 0.872, and, when distilled from white roses, down to 0.811, on account of the presence of up to 85% of stearoptene. The latter type of otto is, of course, of little value from the point of view of odour. Umney has examined a Spanish distillate and finds it to have the following characters:

Sp. gr. 30°/15°	0.844
Optical rotation	-2
Ref. index	1.4565
M. p.	27° to 28°
Percentage of stearoptene	33.3
M. p. of separated stearoptene	31–32°

The writer has pointed out that adulteration with alcohol may be indi-

cated by the alteration in the characters of the oil after washing it with warm water.

The following figures show the effect of such adulteration on the washed oils.

	Original oil			Oil extracted with water			
	d^{15}	n_D^{15}	M. p.	d^{15}	n_D^{15}	M. p.	Geraniol content
1	0.8597	1.46318	19.6°	0.8614	1.46628	18.7°	77.4%
2	0.8547	1.45111	20.2°	0.8622	1.46615	17.5°	68.1%
3	0.8663	1.46565	26°	0.8678	1.46684	26.5°	77.5%

Rosemary Oil.

The limits given in Vol. IV (p. 390) of the physical characters of this oil require slight amendment. English Rosemary oil is frequently slightly lævorotatory. The sp. gr. of genuine Dalmatian oil is sometimes as low as 0.894. The percentage of esters may be as low as 1.2% and of total borneol 9%.

The following figures were determined by the writer and Bennett[1] on samples distilled from plants sent to the writer, so that their authenticity is undisputed.

	1 (Spanish)	2 (French)	3 (French)
Source...	Leaves alone	Leaves and stalks.	Leaves alone.
Sp. gr...	0.917	0.897	0.914
Optical rotation.............................	+5° 30'	−8° 30'	−3°
Esters calculated as bornyl acetate..........	3.2%	3.0%	3.6%
Total borneol................................	19.7%	10.9%	18.5%
Optical rotation of first 10% (100 mm.).....	−1°	−12° 30'	−10°

No. 1 was distilled from herb collected toward the end of last summer and dried in the sun. It consisted entirely of leaves which were well developed and of a fine green colour. The yield was 0.89%.

No. 2 was distilled from stalky herb in the dried condition, collected in February. The proportion of stalks amounted to nearly 60%. Yield of oil 0.4%.

No. 3 was distilled from the same consignment as No. 2, but the stalks were separated and the leaves alone distilled. They yielded 1.09% of oil, containing a high proportion of borneol. The leaves were somewhat discoloured, probably owing to some fermentation having taken place.

The following are the results of fractionation of the above oils:

[1] *Chem. and Druggist*, 1906, 1, 671.

NO. 1

Fraction		Sp. gr.	Rotation	Ref. index	B. p. commencing at
	%				
1	10	0.884	$-1°$	1.4676	152°
2	10	0.890	$-1°$ 10'	1.4680	156°
3	10	0.895	$-1°$ 20'	1.4681	159°
4	10	0.902	$+1°$	1.4682	162°
5	10	0.903	$+1°$ 20'	1.4683	163°
6	10	0.911	$+2°$	1.4686	165°
7	10	0.922	$+2°$ 30'	1.4700	169°
8	10	0.940	$+3°$	1.4736	177°
Residue	20	Partially crystallised		1.4885	185°

NO. 2

Fraction		Sp. gr.	Rotation	Ref. index
	%			
1	10	0.874	$-12°$ 30'	1.4660
2	10	0.878	$-13°$	1.4670
3	10	0.879	$-13°$ 30'	1.4670
4	10	0.883	$-12°$ 20'	1.4670
5	10	0.886	$-11°$ 20'	1.4670
6	10	0.891	$-10°$ 30'	1.4670
7	10	0.896	$-8°$ 30'	1.4678
8	10	0.909	$-5°$ 30'	1.4702
Residue	20			1.4859

NO. 3

Fraction		Sp. gr.	Rotation	Ref. index
	%			
1	10	0.885	$-10°$	1.4660
2	10	0.888	$-10°$	1.4680
3	10	0.891	$-9°$ 20'	1.4685
4	10	0.896	$-7°$ 70'	1.4686
5	10	0.900	$-6°$ 70'	1.4686
6	10	0.909	$-4°$ 50'	1.4686
7	10	0.921	$-1°$	1.4686
8	10	0.938	$-2°$	1.4697
Residue	20	Partially crystallised		

Santalwood Oil.

The following is a complete list of the constituents of santalwood oil hitherto isolated:

Isovaleric aldehyde

Santene.—C_9H_{14} (sp. gr. 0.869; rotation $-0°16'$).

Nortricycloeksantalane, $C_{11}H_{18}$ (sp. gr. 0.913; rotation $-24°$).

Santenone.—$C_9H_{14}O$ (m. p. 48–52°).

Norisoborneol.—$C_9H_{15}.OH$ (m. p. 58–62°).

Teresantalol.—$C_{10}H_{15}.OH$ (m. p. 112–114°).

Nortricycloeksantalal.—$C_{11}H_{16}O$ (sp. gr. 0.997).

Santalone.—$C_{11}H_{16}O$ (sp. gr. 0.991; rotation $-62°$).

A Ketone.—$C_{11}H_{16}O$.

Santalene.—$C_{15}H_{24}$ (two isomers of this sesquiterpene are present).

α-Santalol.—$C_{15}H_{24}O$ (sp. gr. 0.9788; rotation $+1°$ 13′).

β-Santalol.—$C_{15}H_{24}O$ (sp. gr. 0.9728; rotation $-41°$ 47′).

Santalal.—$C_{15}H_{22}O$.

Teresantalic acid.—$C_{10}H_{14}O_2$.

Santalic Acid.—$C_{15}H_{22}O_2$.

Three samples of santalwood oil produced in Mauritius were found to have the following characters:

	Heart-wood oil		Sap-wood oil
	1	2	3
Sp. gr.	0.979	0.982	0.981
Rotation	$-21° 50'$	$-20° 55'$	$-21° 15'$
Ref. index	1.5070	1.5065	1.5067
Acid value	1.9	1.9	1.9
Ester value	5.6	5.6	5.6
Total santalol	96.4%	96.4%	95.8%

Recent adulterations of santalwood oil are glyceryl acetate, benzyl alcohol and castor oil.

Glyceryl acetate is indicated by the high sp. gr., low rotation and high ester value, as well as by the high amount of esters which can be extracted by shaking the oil with 5% alcohol. Benzyl alcohol can be detected by fractional distillation, as it boils at about 205°. The santalol value of samples containing benzyl alcohol will usually be an impossible one—over 100%. Castor oil raises the ester value of the oil and interferes with the solubility in petroleum ether.

Thyme Oil.

Spanish thyme oil contains from 30 to 60% of phenols, mostly carvacrol. It is often mixed with French oil to increase the lower phenol content of the latter.

In reference to oils of origanum, which are naturally dealt with under thyme oil, it should be noted that the various species of this plant have been described so loosely by different botanists that the mere specific name is valueless without the addition of the name of the authority. Thus there are three different species bearing the name *Origanum creticum,* and two each described as *O. hirtum,* and *O. smyrnaeum.* The species from which the oil has been actually distilled and examined are as follows:

> *Origanum majoranoides*—Willd.
> *Origanum onites*—Linn.
> *Origanum maru*—Linn.
> *Origanum hirtum*—Link.

The oil from *Origanum majoranoides* Willd contains a large amount of

phenols, consisting almost entirely of carvacrol. The oil has the following characters:

Sp. gr.	0.962 to 0.9685
Rotation	0° to +1°
Phenols	70–80%

It is soluble in 2.5 to 3 volumes of 70% alcohol. The oil from *Origanum onites* has a sp. gr. 0.948, rotation −10° 15', and phenol value 68%.[1]

The characters of the oil of *Origanum hirtum* are doubtful, but according to recent investigations it contains thymol and no carvacrol, although Jahns in 1879[2] stated that this oil contained carvacrol as its chief constituent. It is probable that Jahns' oil was misdescribed. A sample examined by the Imperial Institute[3] had a sp. gr. 0.944, optical rotation +0° 24', and phenol value 66–67%. Three distillates from *Origanum hirtum albiflorum Hassk.* had the following characters:

Sp. gr.	0.923–0.940
Rotation	+0° 6' to 0° 8'
Ref. index	1.4939 to 1.5044
Phenols	51–60%

Turpentine Oil.

The literature of this oil during the past few years has been very voluminous, but the greater part of it is of an academic nature and has but little bearing on the commercial oil of turpentine used in the English-speaking countries. A number of these publications are quoted for reference, only those of a practical nature being here referred to in detail.

Vèzes has, under the authority of the Executive Committee of the White Cross Congress held in Paris in 1909, suggested the following standards for commercial oil of turpentine.

Turpentine oil is the exclusive product of the aqueous distillation (distillation with water or non-superheated steam) of the turpentine derived from various species of *Pinus*. It is a colourless, often slightly yellowish or greenish, liquid, very mobile, with a characteristic odour. Under a normal pressure of 760 mm., the oil begins to boil between 152 and 156°; at least 80% by weight must have passed over at 164°. The oil should be neutral or give only a faint acid reaction; the permissible acid-content, estimated with phenolphthalein as an indicator, must not exceed 1.5 grm. pure potassium hydroxide (KOH) for every kilogram of oil. The oil must also be free from mineral oils and, in fact, from all bodies other than those generated in the course of the aqueous distillation of turpentine. It may contain small proportions of resin-oil and colophony, resulting from the process of manufacture, but the aggregate weight of these may not exceed 2.5%.

The oil from *Pinus maritima* (France, Spain, Portugal) is lævorotatory,

[1] *Bull. Imp. Inst.*, 1911, 9, 388.
[2] *Arch. d. Pharm.*, 215, 1.
[3] *Bulletin*, 1911, 9, 383.

α_D —29 to —33°; $d_{25°}$ not below 0.8575. The oil from *Pinus halepensis* (Greece, Algeria, Provence) is dextrorotatory, α_D +38, to +41°; $d_{25°}$ not below 0.8550. The American oils, which are obtained from different species of *Pinus* (*P. palustris; P. heterophylla* and others) are partly dextro- and partly lævorotatory; the rotation is very variable, but never higher than in the European oils. $d_{25°}$ not below 0.8560.

The writer has examined two authentic samples of Greek turpentine, an oil used largely in the south of Europe, both for legitimate purposes and for adulterating essential oils, and found them to have the following characters:

	I	II
$d^{15°}$	0.8605	0.862
α_D	+36° 45′	+39°
$n_D^{20°}$	1.4690	1.4736
Commences to distil at	156°	156°
Fraction 156 to 160°	70 %	72 %
α_D of 156 to 160° fraction	+37° 15′	+40°

During the years 1911–1912 an enormous amount of adulteration of American turpentine with wood or stump turpentine was taking place. At the same time great dissatisfaction was expressed at the inferior nature of the Russian turpentine as found on the London market. The present writer was asked to examine a large number of samples with a view to attacking the unsatisfactory position of these oils in the market, and the following details are taken from two papers, "Turpentine Standards" (*Chem. and Druggist*, Aug. 24, 1912) and "Russian Oil of Turpentine" (*Chem. and Druggist*, Oct. 26, 1912) by Ernest J. Parry.

The United States Department of Agriculture last year issued suggested standards for No. 1 or "standard" turpentine as follows: Sp. gr. at 20°= 0.862 to 0.870; ref. index at 20°=1.4680 to 1.4760; 95% should distil below 170°. On polymerisation with sulphuric acid (38 times normal) the residue should not exceed 1% and should have a ref. index of 1.500 to 1.520. There is no particular objection to take to these figures, other than to say that a considerable amount of stump turpentine may be present in an oil complying with them.

The close similarity in physical characters between pure gum turpentine and the so-called stump turpentine, therefore, renders it necessary that some further distinctive features shall, if possible, be taken into account, and the result of the examination of a very large number of pure and adulterated samples led Parry to consider that the behaviour of the oil toward the halogen elements gives the most useful indications of admixture with wood or stump turpentine which, by the way, must be so described in America, where it is not allowed to be dealt in as "turpentine" without proper qualification.

Both the iodine and the bromine values have been recommended in this respect, but the iodine value is, in his opinion, the more useful of the two. Its value depends on the fact that the hydrocarbons and certain other bodies

present in wood turpentine—probably on account of the method of treatment adopted in its manufacture—appear to be more saturated than those present in normal turpentine, and therefore absorb less iodine to form a fully saturated compound.

The iodine value may be determined either by the Wijs or the Hübl method, the figures of course not being identical. The following processes · may be used for the determination of the bromine value: (1) 1 c.c. of the oil is dissolved in 5 c.c. of chloroform, and a 3% aqueous solution of bromine added with shaking until a permanent colouration remains; the strength of the bromine solution is determined in the usual manner, and the amount combining with the oil can be calculated. (2) 1 c.c. of the oil is dissolved in 50 c.c. of absolute alcohol, and 5 c.c. of hydrochloric acid added. A solution of 28 grm. of bromate of potassium and 100 grm. of bromide of potassium per litre is then added until a permanent brown colour remains for 1 minute after well shaking. The bromine absorbed is calculated for 1 c.c., which can be reduced to the proper bromine number by dividing it by the sp. gr., say 0.86.

Parry considers that the halogen absorption value of the 10% left after distillation of 90% of the sample affords very reliable information as regards the presence of wood turpentine, the iodine value for this fraction (Wijs) being about 355 for pure turpentine and only about 250 to 290 for wood turpentine. He gives the following figures for the various fractions of a pure and of adulterated oils.

	(1) Pure American turpentine	(2) Wood turpentine	(3) 5% each (1) and (2)	(4) "Petroleum" turpentine	(5) 50% each (1) and (4)
Sp. gr.	0.886	0.873	0.869	0.808	0.838
Ref. index	1.4720	1.4745	1.4737	1.4490	1.4610
Initial boiling point	155°	159°	157°	98°	99°
Distillate under 160°	74%	61%	68%	48%	62%
Distillate under 170°	94%	78%	84%	75%	82%
Bromine value	2.2	1.46	1.82	0.05	1.1
Iodine value (Hübl)	372	264	321	9.0	190.5
Iodine value (Wijs)	350	240	298	8.4	179
Iodine value of last 10% (Hübl)	360	251	304	9.0	184
Iodine value of last 10% (Wijs)	355	242	298	8.5	177
Ref. index of first 20%	1.4719	1.4731	1.4728	1.448	1.4660
Ref. index of second 20%	1.4700	1.4730	1.4720	1.449	1.4675
Ref. index of third 20%	1.4712	1.4734	1.4722	1.4481	1.4721
Ref. index of fourth 20%	1.4712	1.4732	1.4721	1.4470	1.4721
Ref. index of fifth 20%	1.4781	1.4842	1.4821	1.4495	1.4735

and proposes the following limits for the characters of genuine American turpentine oil:

Sp. gr. at 15° .... 0.862 –0.870
Ref. index 1.4680–1.4730
Initial boiling point .... 154°–155.5°
Distillate under 160° 72–74.5%
Distillate under 170° 95–97.5%
Bromine value 1.96–2.31
Iodine value (Hübl) 360–375
Iodine value (Wijs) 335–350
Iodine value of last 10% (Hübl) 349–369

Iodine value of last 10 % (Wijs)	350–365
Ref. index of first 20 %	1.4700–1.4722
Ref. index of second 20 %	1.4700–1.4724
Ref. index of third 20 %	1.4710–1.4735
Ref. index of fourth 20 %	1.4710–1.4740
Ref. index of fifth 20 %	1.4780–1.4821

The results of Parry's investigations in regard to Russian turpentine confirm the fact, which is stated by Professor Schindelmeiser of Dorpat University, that most of the Russian turpentine oil of commerce has had the early runnings removed for home consumption, later fractions being then exported.

The following figures are those of a number of Russian turpentines on the London market, and which are, subject to the fact that no large quantity can be obtained that has not had its earlier fractions removed, accepted as satisfactory:

	1	2	3	4
Initial b. p.	157°	156°	157°	158
Distils below 155°	none	none	none	none
Distils 155°–160°	1 %	1 %	5 %	11 %
Distils 160°–165°	44 %	45 %	40 %	18 %
Distils 165°–170°	37 %	35 %	42 %	48 %
Distils 170°–180°	15 %	16 %	10 %	19 %
Distils above 180°	3 %	3 %	3 %	4 %
Sp. gr. at 15°	0.863	0.8635	0.863	0.868
Ref. index at 20°	1.4730	1.4726	1.4725	1.4748
Optical rotation	+4°28′	+4°30′	+9°	+8°
Absorbed by 5 % KOH	nil	nil	nil	nil

A very large number of samples, however, have been even more largely deprived of their early runnings, and contain a considerable amount of hydrocarbons boiling over 180°, and also a considerable amount of acid bodies, which are absorbed by potassium hydroxide. Such samples are quite useless to the rectifier, as their redistillation must ensure the removal of the acid bodies and also of the bodies boiling over 180°, with a resulting loss which causes the rectification to be unremunerative.

The following are typical samples of this type:

	1	2	3	4
Initial b. p.	148°	146°	146°	153°
Distils below 155°	2 %	3 %	5.5 %	1 %
Distils 155°–160°	3 %	3 %	5 %	3 %
Distils 160°–165° } Distils 165°–170° }	35 %	34 %	22 %	36 %
Distils 170°–180°	48 %	50 %	46 %	50 %
Distils above 180°	12 %	10 %	21.5 %	10 %
Sp. gr. at 15°	0.868	0.8665	0.878	0.869
Ref. index at 20°	1.4762	1.4756	1.4780	1.4792
Optical rotation	+8°	+9°	+11°	+12°45′
Absorbed by KOH	6 %	8.5 %	7 %	3 %

Parry gives the following figures for two samples of virgin crude Russian turpentine:

	I	II
Sp. gr.	0.867	0.865
Optical rotation	+7°50′	+10°
Ref. index	1.4718	1.4736
Absorbed by 5 % KOH	5 %	6 %
Distilled below 155°	traces only	traces only
Distilled 155°–160°	65 %	63 %
Distilled 160°–165°	11 %	9 %
Distilled 165°–170°	13 %	15 %
Distilled 170°–180°	7.5 %	7 %
Distilled above 180°	3.5 %	6 %

From these two samples the tarry and acid bodies were removed and the rectified sample, in the case of No. I had the following characters:

Sp. gr.	0.8646
Optical rotation.	+8°
Ref. index.	1.4890
Absorbed by KOH.	none
Distils below 155°.	68 %
Distils 155°–160°.	none
Distils 160°–165°.	13 %
Distils 165°–170°.	10 %
Distils 170°–180°.	7 %
Distils above 180°.	2 %

For an exhaustive account of the characters and minute analysis of wood turpentine the *Bulletin of the U. S. Department of Agriculture* (No. 105, 1913) by L. F. Hawley should be consulted. On the polymerisation of turpentine by sulphuric acid see J. H. Coste,[1] and Eiber and Hue.[2] The *Bulletin of the U. S. Department of Agriculture* (No. 144, 1911) by Veitch and Donk on wood turpentine should also be consulted.

Wintergreen Oil.

True wintergreen oil is the product of the distillation of the leaves of *Gaultheria procumbens*, but the oil from the bark of *Betula lenta* is so closely identical with it that the two are used more or less indifferently, except in so far as the latter is much less expensive than the former, and so finds a larger employment. Both oils consist almost entirely of methyl salicylate, their characters being as follows:

	Gaultheria oil	Betula oil
Sp. gr.	1.180–1.187	1.180–1.187
Ref. index.	1.5350	1.5350
Distils between.	218°–221°	218°–221°
Rotation.	under–1°	0°
Methyl salicylate.	98–99 %	98–99 %

The principal adulterant is synthetic methyl salicylate. The following colour test, although by no means absolute will give useful indications in cases of gross adulteration with synthetic methyl salicylate.[3]

The application of the test is as follows:

To 5 drops of oil in a test-tube add 5 drops of a 5% alcoholic solution of vanillin and 1 c.c. of alcohol. Shake well, and add 2 c.c. of concentrated sulphuric acid and mix thoroughly.

The following table indicates the results of the examination of typical samples:

Colour produced

Oil of *Gaultheria procumbens*	Intense crimson.
Oil of *Betula lenta*	Deep blood red.
Doubtful (a).	Reddish brown.
Doubtful (b).	Reddish brown.
Methyl salicylate (synthetic).	Yellow.

By this intensity of colouration there can be no question that one can see

[1] *Analyst*, 1910, 35, 112.
[2] *Chem. Zeit.*, 1910, 34, 643, 657.
[3] *Perfumery Record*, 1914, Vol. 4.

a difference between oils that are pure and oils that are grossly adulterated, but whether it can be made into an accurate colorimetric test is, of course, difficult to say.

If traces of chlorine are found in the oil (see under Oil of Almonds) a crude artificial methyl salicylate must have been used as the adulterant.

American Wormseed Oil.

E. K. Nelson[1] has published an exhaustive investigation on the constitution of ascaridol, which appears to be the active constituent of this oil.

The hitherto isolated constituents are p-cymene, sylvestrene, d-camphor and ascaridol.   The oil has the following characters:

Sp. gr. 0.965–0.990
Optical rotation. .... −4° to −8° 50′
It is soluble in 3 vol. of 70% alcohol.

Ylang-ylang Oil.

The tree whose flowers yield this much valued oil is *Cananga odorata*, which grows to perfection in the Philippine Islands, more especially in Manila. The oil produced in Java and the neighbourhood is of far less value and is known as Cananga oil.   The reason for the difference between these two commercial varieties is still doubtful, as there is every reason to believe that the tree is identical in both cases.

The constituents of the oil so far identified are as follows: linalol, geraniol, cadinene, *para*cresol methyl ether, benzoic and acetic esters, pinene, eugenol methyl ether, isoeugenol, methyl salicylate, and benzyl esters.   Formic acid esters, safrole, isosafrole, nerol and farnesol are also probably present in the oil.

The physical characters of the oil vary within very wide limits and organoleptic tests are very necessary in its valuation.   The two oils have the following characters:

	Manila	Madagascar	Bourbon
Sp. gr.	0.922–0.970	0.960–0.981	0.960–0.975
Rotation..................................	−27° to −52°	−30° to −42°	−35° to −43°
Ref. index...............................	1.491–1.506	1.496–1.513	1.499–1.515
Ester value..............................	75–155	110–175	131–162
Non-volatile (2 hours at 212°F.).........	4–16.5%	15–30%	22–43%

Cananga Oils.

Sp. gr. 0.900–0.950
Rotation. .... −17°–55°
Ref. index. 1.4950–1.5110
Ester value. 30–100

These oils should be soluble, with at most slight opalescence in 0.5 to 2 volumes of 90% alcohol, becoming turbid by further addition of alcohol.

[1] *J. Amer. Chem. Soc.*, 1913, **35**, 84.

Petroleum oil is a common adulterant of cananga oil and will, apart from altering the physical character above quoted, decrease the solubility. Benzyl benzoate will be indicated by a high fixed residue on the water-bath after 2 hours heating.

In a communication to the *Philippine J. Sci.*, in 1908, R. F. Bacon, who has studied the production of ylang-ylang oil in Manila in much detail, and has himself superintended the distillation of certain of the samples upon which he reports, gave as the figures from his examinations the following:

	First quality	Second quality
Sp. gr. 30°/40°	0.911–0.958	0.896–0.942
Optical rotation (30°)	−27° to −49°	−27° to −87°
Ref. index (30°)	1.4747–1.4940	1.4788–1.5082
Ester number	90–138	42–94

At a later date, in a communication to the same journal he confirmed his classification, and presented figures for the examination of a further number of oils. He gave as limits for first and second grade oils the following:

	First quality	Second quality
Sp. gr.30°/40°	0.910–0.945	c.905–0.0925
	(mostly 0.920–0.920)	(mostly 0.910–.915)
Optical rotation	−22° to −50°	−38° to 79°
	(mostly − 40° to −50°)	(very irregular).
Ref. index	1.4863–1.4944	1.4910–1.5030
Ester number	92–129	71–88
	(average 104)	(average 81)
Ester number after acetylation	154–214	96–141
	(average 182)	(average 118)

ERRATA IN VOL. IV.

Title pages, ii, iii and viii, for "Ernest C. Parry" read "Ernest J. Parry".

Pages 312 and 313, in several places, "anethol" should read "anethole."

Page 314, line 15 from bottom, "safrol," should read "safrole."

Page 358, lines 16 and 17 should be deleted. Line 18 "Solway" should read "Salway;" and insert after "2037," see also, *Trans. Chem. Soc.*, 1908, 93, 1653."

Page 359, line 9, for "$C_{11}H_{12}O_3$" read "$C_{13}H_{14}O_3$" and add after "myristian," line 10, "see Power and Salway, *Trans.*, 1907, 91, 2039 and 1908, 93, 1653.

Page 369, line 1 below table, for "apiol" read "apiole."

Page 389, line 9 from bottom, for "Schimmell" read "Schimmel."

Page 429, line 18, for "safrol" read "safrole."

Page 433, in last column of table "anethol" should read "anethole" and "safrol" should read "safrole."

Page 434, under *Asarum Europaeum*, it should be noted that asarol has been shown to be identical with *d*-linalol (Power and Lees, *Trans.*, 1902, 91, 63).

Page 445, In table under "Hedeoma," delete in last column "Hedeomol, pulegone" and replace by "See page 377." Under "Hop Oil" add in last column, "For later work, see "Chapman, *Trans.*, 1903, 93, 505."

Page 450, last column, insert "see however page 358 for latest work.

Page 460, in table, for "anethol" read "anethole."

Page 461, in table, for "safrol" read "safrole."

In index page 463 under "acid value" for "Dieterick" read "Dieterich." For "chiconolic acid" read "chironolic acid." For "chrysanthenum" read "chrysanthemum."

Page 464 for "Dieterick" read "Dieterich."

Page 464, for "Heany" read "Heavy," "light comphor oil" read "light camphor oil."

Page 465, for "patchonlene" read "patchoulene." "Rubler" read "rubber." Under "Tiemann" for "cital" read "citral."

TANNINS.

By W. P. DREAPER, F. I. C.

Tannin Materials.—The geographical distribution of tannin in the vegetable world has been dealt with by J. Dekker.[1] Although the number of plants containing tannin is relatively small this substance appears in all the groups or subdivisions adopted by botanists. Algæ, fungi, and lichens often contain it, but the mass of raw material available in such cases is not sufficient to have any commercial value. Very few of the mosses give a positive indication for tannin. Many of the ferns contain tannin, from mere traces to 10%, but it is in the higher group of seed plants that tannin occurs abundantly from a commercial point of view. Among the *Gymnosperms* a number of plants contain tannin, notably the pine, hemlock, spruce and fir. The *Dicotyledons* furnish the largest number of plants rich in tannin. Several of this order are widely distributed from the tropics to the limits of vegetation. The ones used in commerce are chiefly tropical. Thus the *Combretaceæ*, consisting of about 240 tropical species, yield from one of them the myrabolans of commerce. The *Rhizophoraceæ* yield the mangrove bark. The *Leguminosæ* which are chiefly useful are also tropical such as the wattle, algarobilla, ratanhia, kino, and divi-divi. Generally speaking the chief commerical sources of tannin are found between the parallels of 30° North and South latitude, an exception is found in the *Fagaceæ* which contain the oaks and chestnuts.

Nature of material	Percentage of tannin
Mangrove bark, African	28.4
Mangrove bark, East African	28–42
Mimosa Bark, Australian	30
Mimosa bark, S. African	35–40
Mimosa bark, Paraguay	22–23
Quebracho Wood, Argentine	18.7
Algarobilla pods, Chilian	45.4
Myrabolans	29.3
Myrabolans another sample	38–40
Valonia	31–32
Oak bark, German	11.2
Oak bark, Californian	11.4
Canaigre, Texas	18.6
Molle Bark, Argentine	14.1
Chestnut, Italian	7–9
Sumac, Sicilian	25–29
Lentiscus	10–15
Mexico Bark (Lysilonia Candida)	24
Mallet Bark	45.7
Fir Bark	13–16
Knoppern	34–40
Trillo	40
Larch bark, German	8–10

[1] De looistoffen, *Bull. Koloniaal Museum te Haarlem*, 1906, No. 35.

With the possible exceptions of sumac and canaigre, these tannin plants are not cultivated. The tannin content of several of these materials (barks, nuts and wood) is shown in the foregoing table of recent estimations by Parker, Pollak, Levi and Orthman and others, the assay being conducted by the official hide powder process. Other results will be found under different headings.

Constitution of Tannins.—E. Fischer[1] claims that gallo-tannins are acyl compounds of glucose but M. Nierenstein[2] does not accept this view, as the result of a further examination of the tannin of hemlock, knoppern and some other tannins which contain no sugar.

Ellagic Acid.—By electrolytic reduction Nierenstein and Rixon[3] have produced leucoellagic acid which can also be obtained from digallic acid.[4] It has no tanning properties. If reduction takes place at 70° instead of at ordinary temperature pentahydroxydiphenylmethylolide is produced, and at 100°, a series of hexahydroxydiphenyls.

Tannin (gallotannic acid).—The evidence of the composite nature of gallo-tannic acid and therefore possibly of the other tannins, is accumulating and becoming more definite. Walden[5] first came to this conclusion after studying the dialytic behaviour and the optical activity of the acid as prepared by different methods. Aweng[6] came to the same conclusion through studying the condensation products with formaldehyde and Kunz-Krause and Nierenstein have since independently confirmed this view. The acid character of gallotannic acid as expressed by the presence of free hydroxyl groups (Schiff's formula) is not accepted by many chemists, notably by Boettinger.[7] Paniker and Stiasny have more recently studied the question of the acid character of gallotannic acid by determining the hydrion concentration of gallotannin by Bredig and Fraenkel's diazoacetic ester method.[8] The results obtained seem to confirm the opinion that gallotannic acid is a mixture of two or more chemical individuals, possibly closely allied.

To prepare pure gallotannic acid, the last investigators consider that the method entailing the separation of sodium gallate through its insolubility in acetic ether, is the best available. Even after salting out with sodium chloride twenty-four times, gallic acid was still present, and the method utilising the unequal distribution of the acids in ether and acetone also gave unsatisfactory results.

Analysis of Tannins.—Since the publication of Volume V the American Official Method of estimating Tannins has been so modified that it is necessary to redescribe it in detail.

It now stands as follows:

[1] *Zeit. Angew Chem.*, 1913, 26, 547.
[2] *Chem. Zeit.*, 1913, 37, 1237.
[3] *Collegium*, 1913, 514, 53.
[4] *Collegium*, 1912, 202.
[5] *Ber.*, 1897, 30, 3151.
[6] *Rev. Intern. Falsific.* 1898, 11, 29.
[7] *Ber.*, 1884, 17, 1503.
[8] *Zeit. Phys. Chem.*, 50, 202.

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OFFICIAL METHOD OF THE AMERICAN LEATHER CHEMISTS ASSOCIATION (A. L. C. A.) FOR THE ANALYSIS OF VEGETABLE MATERIALS CONTAINING TANNIN (1914).

I. Raw and Spent Materials.

(1) **Caution.**—Proper care must be taken to prevent any change in the water content of raw materials during the sampling and preliminary operations. . (See "General" under Sampling.)

(2) **Preparation of Sample.**—The sample must be ground to such a degree of fineness that the entire sample will pass through a sieve of 20 meshes to the inch (linear).

(a) The temperature used for drying samples of spent material for grinding must not exceed 60° C.

(b) Samples of raw material too wet to be ground may be dried before grinding as in (a). In this case a preliminary determination of water must be made according to (IV) on the sample as received. If the portion of the sample taken for the water determination is in pieces too large to dry properly, it is permissible to reduce these to smaller size as rapidly and with as little loss of water as possible. .

(3) **Water Determination.**—10 grm. of the ground material shall be dried in the manner and for the period specified for evaporation and drying in extract analysis (see IV).

(4) **Amount of Sample to be Extracted.**—Such an amount of raw material shall be extracted as will give a solution containing as nearly as practicable 0.4 grm. tannin to 100 c.c. (not less than 0.375 or more than 0.425). Of spent materials such an amount shall be taken as will give a solution of as nearly as practicable the above concentration.

(5) **Extraction.**—Extraction shall be conducted in an apparatus consisting of a vessel in which water may be boiled and a container for the material to be extracted. This container shall be provided above with a condensation chamber so arranged that the water formed from the condensed steam will drip on the material to be extracted, and provided below with an arrangement of outlets such that the percolate may either be removed from the apparatus or be delivered to the boiling vessel. The boiling vessel must be so connected that it will deliver steam to the condensation chamber and that it may receive the percolate from the container. The condensation water from the condenser must be at approximately the boiling temperature when it comes in contact with the material to be extracted.

The material of which the boiling flask is composed must be inert to the extractive solution. Suitable provision must be made for preventing any of the solid particles of the material from passing into the percolate.

(A) *Woods, Barks, and Spent Materials.*—500 c.c. of the percolate shall be collected outside in approximately 2 hours and the extraction continued with 500 c.c. for 14 hours longer by the process of continuous extraction with

reflux condenser. The applied heat shall be such as to give by condensation approximately 50 c.c. in 1½ hours.

(B) *Materials Other than Woods, Bark and Spent.*—Digest the material in the extracter for 1 hour with water at room temperature and then extract by collecting 2 litres of percolate outside in approximately 7 hours.

(6) **Analysis.**—The percolate shall be heated at 80° C., be cooled, made to the mark and analysed according to the official method of extracts.

II. Analysis of Extracts.

(7) **Amount and Dilution for Analysis.**—(A) *Fluid Extracts.*—Fluid extracts shall be allowed to come to room temperature, be thoroughly mixed, and such quantity weighed for analysis as will give a solution containing as nearly as possible 0.4 grm. tannin to 100 c.c. (not less than 0.375 nor more than 0.425). Precautions must be taken to prevent loss of moisture during weighing. Dissolve the extract by washing it into a litre flask with 900 c.c. of distilled water at 85° C.

Cooling.—(a) The solutions prepared as above shall be cooled rapidly to 20° C. with water at a temperature of not less than 19° C., be made to the mark with water at 20° C. and the analysis proceeded with at once, or

(b) The solution shall be allowed to stand over night, the temperature of the solution not being permitted to go below 20° C., be brought to 20° C. with water at not less than 19° C., be made to the mark with water at 20° C. and the analysis proceeded with.

(B) *Solid and Powdered Extracts.*—Such an amount of solid or powdered extract as will give a solution of the strength called for under liquid extracts shall be weighed in a beaker with proper precautions to prevent change of moisture. 100 c.c. of distilled water at 85° C. shall be added to the extract and the mixture placed on the water-bath, heated and stirred until a homogeneous solution is obtained. When dissolved, the solution shall immediately be washed into a litre flask with 800 c.c. of distilled water at 85° C., be cooled, etc., as under (A) above.

NOTE.—It is permissible to make up 2-litre instead of 1-litre solutions, dissolving by washing into flask with 1,800 c.c. water at 85° C. in case of fluid extracts and 1,700 c.c. water at 85° C., in case of solid or powdered extracts.

(8) **Total Solids.**—Thoroughly mix the solutions; pipette 100 c.c. into tared dish, evaporate and dry as directed under "Evaporation and Drying." (See IV.)

(9) **Water.**—The water content is shown by the difference between 100% and the total solids.

(10) **Soluble Solids.**—S. & S. No. 590, 15 cm. single, pleated, filter paper shall be used for the filtration.

The kaolin used shall answer the following test: 2 grm. kaolin digested with 200 c.c. of distilled water at 20° C. for 1 hour shall not give more than 1

mg. of soluble solids per 100 c.c., and shall be neutral to phenolphthaleīn.   To
1 grm. kaolin in a beaker add sufficient solution to fill the paper, stir and pour
on paper.   Return the filtrate to the paper when approximately 25 c.c. has
collected, repeating operation for 1 hour, being careful to transfer all kaolin
to the paper.   At the end of the hour remove the solution from the filter
paper, disturbing the kaolin as little as possible.   Bring so much as needed of
the original solution to exactly 20° C. as described under (7), refill the paper
with this solution and begin to collect the filtrate for evaporating and drying
so soon as it comes clear.   The paper must be kept full and the tempera-
ture of the solution on the filter must not fall below 20° C. nor rise above
25° C. during this part of the filtration.   The temperature of the solution
used for refilling the paper must be kept uniformly at 20° C. and the funnels
and receiving vessels must be kept covered.

   Pipette 100 c.c. of clear filtrate into tared dish; evaporate and dry as under
(8).

   (11) **Insolubles.**—The insoluble content is shown by the difference between
the total solids and the soluble solids, and represents the matters insoluble in
a solution of the concentration used under the temperature conditions
prescribed.

   (12) **Non-tannins.**—The hide powder used for the non-tannin determina-
tion shall be of woolly texture well delimed and shall require between 12
and 13 c.c. of $N/10$ NaOH to neutralise 10 grm. of the absolutely dry powder.

   (a) Digest the hide powder with ten times its weight of distilled water till
thoroughly soaked.   Add 3% of chrome alum $Cr_2(SO_4)_3, K_2SO_4, 24H_2O$, in 3%
solution, calculated on the weight of the air-dry powder.   Agitate frequently
for several hours and let stand over night.   Squeeze and wash by digesting
with four successive portions of distilled water, each portion equal in amount
to fifteen times the weight of the air-dry powder taken.   Each digestion
shall last for 15 minutes, and the hide powder shall be squeezed to approxi-
mately 75% water after each digestion except the last, a press being used if
necessary.   The wet hide powder used for the analysis shall contain as nearly
as possible 73% of water, not less than 71% nor more than 74%.   Determine
the moisture in the wet hide powder by drying approximately 20 grm.   (See
IV.)   To such quantity of the wet hide as represents as closely as practicable
12.5 grm. (not less than 12.2 nor more than 12.8) of absolutely dry hide add
200 c.c. of the original analysis solution and shake immediately for 10 minutes
in some form of mechanical shaker.   Squeeze immediately through linen,
add 2 grm. of kaolin (answering test described under (9)) to the detannised
solution and filter through a single-folded filter (No. 1F Swedish recom-
mended) of size sufficient to hold the entire filtrate, returning until clear.
Pipette 100 c.c. of filtrate into a tared dish, evaporate and dry as in (8).

   The weight of the non-tannin residue must be corrected for the dilution
caused by the water contained in the wet hide powder.

   Funnels and receiving vessels must be kept covered during filtration.

Flasks graduated to deliver 200 c.c. are recommended for measuring the analysis solution to be detannised.

(b) Digest the hide powder with the amount of water and add the amount of chrome alum in solution directed under (a).

Agitate in some form of mechanical shaker for 1 hour and proceed immediately with washing and subsequent operations as directed under (a).

NOTE.—In order to limit the amount of dried hide powder used, determine the moisture in the air-dry powder and calculate the quantity equal to 12.5 grm. of actual dry hide powder. Take any multiple of this quantity according to the number of analyses to be made, and after chroming and washing as directed, squeeze to a weight representing as nearly as possible 73% of water. Weigh the whole amount and divide by the multiple of the 12.5 grm. of actual dry hide powder taken to obtain the weight of wet hide powder for 200 c.c. of solution.

(13) **Tannin.**—The tannin content is shown by the difference between the soluble solids and the corrected non-tannins, and represents the matters absorbable by hide under the conditions of the prescribed methods.

III. Analysis of Liquors.

(14) **Dilution.**—Liquors shall be diluted for analysis with water at room temperature so as to give as nearly as possible 0.7 grm. solids per 100 c.c. of solution. Should a liquor be of such character as not to give a proper solution with water of room temperature it is permissible to dilute with water at 80° C. and cool rapidly as described under (7, A, a).

(15) **Total Solids.**—To be determined as in Extract Analysis.

(16) **Soluble Solids.**—To be determined as in Extract Analysis.

(17) **Insolubles.**—Determined as in Extract Analysis.

(18) **Non-tannins.**—To be determined by shaking 200 c.c. of solution with an amount of wet chromed hide powder, containing as nearly as possible 73% water, corresponding with an amount of dry hide powder shown in the following table:

Tannin range per 100 c.c.	Dry powder per 200 c.c.
0.35—0.45 grm.	9.0—11.0 grm.
0.25—0.35 grm.	6.5— 9.0 grm.
0.15—0.25 grm.	4.0— 6.5 grm.
0.00—0.15 grm.	0.0— 0.0 grm.

Solutions to be shaken for non-tannins as in Extract Analysis and 100 c.c. evaporated as in Extract Analysis.

IV. Temperature, Evaporation and Drying, Dishes.

(19) **Temperature.**—The temperature of the several portions of each solution pipetted for evaporating and drying, that is, the total solids, soluble solids and non-tannins must be identical at the time of pipetting.

(20) **Evaporation.**—All evaporations and dryings shall be conducted in the form of apparatus known as the "Combined Evaporator and Dryer" at a temperature not less than 98° C. The time of evaporation and drying shall be 16 hours.

(21) **Dishes.**—The dishes used for evaporation and drying of all residues shall be flat-bottomed glass dishes of not less than 2¾ in. diameter nor more than 3 in. in diameter.

V. Determination of Total Acidity of Liquors.

(17) **Reagents.**—(a) 1% solution of gelatin neutral to hæmatin. The addition of 25 c.c. of 95% alcohol per litre is recommended to prevent frothing. If the gelatin solution is alkaline, neutralise with $N/10$ acetic acid and if acid neutralise with $N/10$ sodium hydroxide.

(b) Hæmatin. A solution made by digesting hæmatin in cold neutral 95% alcohol in the proportion of 0.5 grm. of the former to 100 c.c. of the latter.

(c) Acid washed kaolin free from soluble matters.

(d) $N/10$ sodium hydroxide.

Directions.—To 25 c.c. of liquor in a cylinder that can be stoppered, add 50 c.c. of gelatin solution, dilute with water to 250 c.c., add 15 grm. of kaolin and shake vigorously. Allow to settle for at least 15 minutes, remove 30 c.c. of the supernatant solution dilute with 50 c.c. of water and titrate with $N/10$ sodium hydroxide using hæmatin solution as the indicator. Each c.c. $N/10$ sodium hydroxide is equivalent to 0.2% acid as acetic.

VI. General.

(18) When materials containing sulphite-cellulose extract are analysed, the fact that the material contains sulphite-cellulose extract shall be noted on the report.

(19) On public analytical work by members of this Association, the fact that the Official Method has been used, shall be so stated.

The American Official Method of Sampling Tanning Materials has also been altered to the following:

OFFICIAL METHODS FOR SAMPLING TANNING MATERIALS (1914).

General.—Extract, whether liquid or solid, and tanning materials in general all contain moisture. The amount of moisture varies with climatic conditions, but especially in liquid, and in most solid extracts becomes less as the extract is exposed to the air. As the value of any material shown by analysis is directly dependent upon the amount of moisture contained, and as an exposure of a comparatively few moments may alter appreciably the amount of moisture it is apparent that the sampling in all its details should be done as

quickly as consistent with thoroughness and with great care to expose the material as little as possible to the air. The portions taken as samples should be placed at once in containers as nearly air tight as possible, and preferably of glass. Wood, cardboard, poorly glazed crockery, etc., are all porous and more or less absorbent and not suitable for retaining samples.

Liquid extract cannot be accurately sampled when it contains any frozen material. A sample of extract taken after live steam has been run into the extract has not the same concentration as the original extract. A sample of spent bark which has been standing where dust from fresh ground bark has sifted into it does not represent the degree of extraction of the spent bark. Samples of the liquor which have been kept with no preservative in them for some time do not represent the condition of the liquor when sampled.

(1) **Number of Packages to be Sampled.**—When carload lots, or less, of bags are to be sampled, 7% of the number of bags shall be sampled. When shipments of more than a carload and less than 2,000 bags are to be sampled, 20 bags shall be sampled. When shipments of more than 2,000 bags are to be sampled, 1% of the number of bags shall be sampled.

When 70, or less, barrels are to be sampled, 10% of the number of barrels shall be sampled. When from 71 to 140 barrels are to be sampled, 9% of the number of barrels shall be sampled. When from 141 to 210 barrels are to be sampled, 8% of the number of barrels shall be sampled. When from 211 to 280 barrels are to be sampled, 7% of the number of barrels shall be sampled. When from 281 to 350 barrels are to be sampled, 6% of the number of barrels shall be sampled. When from 351 to 420 barrels are to be sampled, 5% of the number of barrels shall be sampled. When from 421 to 500 barrels are to be sampled, 4% of the number of barrels shall be sampled. When more than 500 barrels are to be sampled, 3% of the number of barrels shall be sampled.

(2) **Liquid Extract in Barrels.**—The heads shall be removed from the number of barrels specified in (1), the contents of each barrel stirred until homogeneous, and a sample of equal size taken from each barrel. These sub-samples shall be put together in a suitable closed container and be thoroughly mixed. From this bulk duplicate samples shall be drawn for analysis. These samples shall be preserved in air-tight glass containers, labelled with the date of sampling and such distinguishing marks as may be necessary. When a considerable period of time is likely to elapse between the sampling and the analysis, each individual sample shall be weighed when prepared and the certified weight of the sample be marked on the label.

(3) **Liquid Extract in Bulk.**—The extract shall be agitated with air, be plunged or be mixed by some other efficient means until homogeneous. Equal samples shall then be taken from different parts of the bulk, be placed in a proper container, and be thoroughly mixed and sampled as described in (2).

(4) **Liquid Extract in Tank Cars.**—The following methods are permissible:

(a) The extract shall be unloaded into clean, dry containers and sampled according to (3); or,

(b) The extract shall be mixed until homogeneous, by plunging through the dome or other effective means, then numerous equal samples shall be taken from as widely scattered parts of the bulk as possible. These samples shall then be placed in a suitable container, be mixed and sampled as in (2).

NOTE.—As it is almost impossible to secure a homogeneous mixture of the extract in a tank car, this method should be used only when no other is possible. Or,

(c) The extract shall be sampled as follows while the car is being unloaded: A quart sample shall be taken from the discharge 3 minutes after the extract has begun to run; another quart sample shall be taken 3 minutes before the extract has all run out, and three other quart samples shall be taken at equal intervals between these two. These five samples shall be transferred to a suitable container as soon as taken, be thoroughly mixed and sampled as in (2).

(5) **Solid Extracts.**—The number of packages specified in (1) shall be selected, as nearly as practicable, of equal size. Whenever possible every nth package shall be set aside for sampling while the extract is being unloaded. When this is not possible, the packages shall be selected from as uniformly distributed parts of the bulk as possible.

Samples of as nearly equal size as practicable shall be taken from each package and these samples shall represent as nearly as may be, proportionally the outer and inner portions of the extract. These subsamples shall be placed in a clean, dry closed container. When sampling is completed, the whole composite sample shall be broken up till it will pass through a sieve of 1 in. mesh; it shall be reduced to the required bulk by successive mixings and quarterings. From this bulk duplicate samples of the required size shall be taken, be wrapped in paraffin paper, and be enclosed in the smallest clean, dry air-tight glass receptacles that will hold them, labelled, etc., as in (2).

Sampling at place of manufacture shall be conducted by running a portion from the middle of each strike into a mould holding at least two pounds. These subsamples shall be preserved with proper precautions against evaporation, and be sampled for analysis as above.

(6) **Crude Tanning Materials.**—A. Shipments in bags, mats, barrels or other similar packages.

The number of packages specified in (1) shall be emptied in uniform horizontal layers in a pile on some clean surface. At least five equal samples shall be taken from top to bottom through the pile at uniformly distributed spots. These subsamples shall be mixed together and the bulk be reduced by mixing and quartering to the desired size. Duplicate samples of not less than two quarts each shall be preserved in air-tight containers properly labelled.

When the number of packages to be sampled is so great as to make one

pile impracticable, two or more piles may be made, and the samples from the several piles properly mixed.

B. *Shipments in Bulk.*—(1) Nuts, beans, pods, ground materials, etc

Equal portions shall be taken from at least ten uniformly distributed parts of the bulk, be mixed and sampled as in "A."

(2) Barks, Wood, Etc., in Sticks.

Sticks shall be taken from at least ten uniformly distributed parts of the bulk, be sawed completely through, and the sawdust thoroughly mixed and sampled as in "A."

C. *Materials Prepared for Leaching.*—Samples of equal size shall be taken at uniform intervals as the material enters the leach and be kept in a suitable container till sampling is completed. This bulk shall then be thoroughly mixed, be reduced by mixing and quartering, and duplicate samples for analysis of at least one quart in size be preserved in air-tight containers, as in "A."

(7) **Spent Materials from Leaches.**—Samples of spent materials shall be taken from the top, middle and bottom, and in each case from the centre and outer portions of the leach. These subsamples shall be thoroughly mixed, be reduced in bulk by mixing and quartering, and duplicate samples of at least one quart in size be preserved for analysis.

(8) **Tanning Liquors.**—The liquor shall be mixed by plunging or other effective means till homogeneous and then samples of at least one pint taken for analysis. The addition of 0.03% of thymol or other suitable anti-ferment to the sample is essential to keep the liquor from altering its original condition.

When outline samples are taken from day to day and a composite sample analysed, samples of equal size shall be taken from each vat after thorough mixing, be preserved in covered containers in as cool a place as possible, and kept from fermentation by the addition of suitable anti-ferment, as above. This bulk shall be mixed until homogeneous, and samples of not less than one pint each preserved for analysis. ·

When a sample is taken by a member of this association in accordance with the above method, it is requested that he state both upon the label of the sample submitted and upon the analysis blank, that this sample has been taken in accordance with the official method of sampling of The American Leather Chemists Association.

It would be undoubtedly wise for chemists not working within this association to follow the same method of sampling when working in America.

Precipitation of Tannin.—The use of fat-free casein (Kahlbaum's pure casein) has been suggested by C. W. Spiers[1] for separating tannin from cider. With a 0.5% solution of "pure" tannic acid, 50 c.c. of the solution was shaken with two 1-grm. quantities of casein for 15 minutes. The solution was filtered before the addition of the second portion and finally passed through

[1] *Collegium,* 1914, 530, 358.

barium sulphate. 5 c.c. of the solution, before and after detanning, were titrated with permanganate.

When testing the non-tans filtrate for tannin, Stiasny[1] has recently suggested the use of the following method: 3 c.c. of the solution is taken and 1 c.c. of a saturated salt solution and 2 drops of a 1% solution of meta-phosphoric acid added and then 2 drops of a 5% gelatin 5% salt solution.

Differences in the amount of non-tans obtained have been observed to be due to variations in the hide powder in different samples which actually conform to the official regulations. These absorb varying amounts of non-tans. Stiasny[2] has suggested, to meet this difficulty, that "if two hide powders gave no soluble matter on washing, and if the non-tans solution showed no reaction for tannin, the hide powder giving the higher non-tans should be regarded as correct." It must be remembered, however, that if the error is due to the relative extraction of some substance from the hide powder under the conditions existing during the extraction of the tannin and not to mere water-washing, the reverse might be the more correct procedure. The latter condition may quite possibly be partly responsible for the difference. The Levi-Orthman reagent 33 (a chromium compound), which is stated by the authors to differentiate between a tannin and sulphite-cellulose, has not been accepted unreservedly by the A. L. C. A. owing to differences in the results obtained for tannin as compared with the official method.[3]

A chromed gelatin is suggested to take the place of hide powder by E. Guisiana.[4] The gelatin is tanned in a basic chrome solution consisting of 100 grm. of chrome alum and 15 grm. of sodium carbonate to the litre of water. Thin leaves of pure white gelatin are placed in this solution for 24 hours. The gelatin is then insoluble in hot water. It is washed in water and treated with a 1% solution of ammonia and again well washed and dried between filter paper. The tanned gelatin may be kept in a flask of distilled water. For 10 c.c. of tannin solution the equivalent of 5 grm. of dry gelatin is taken. The shaking, filtration, etc., are as in the official hide powder method.

The analysis of spent bark, nuts, etc., may be illustrated in its results by following figures for oak bark as given by F. H. Small.[5]

	New bark %	Spent bark %
Total solids	24.15	9.73
Soluble	20.75	8.08
Non-tans	8.63	3.64
Tannin	12.12	4.44
Insoluble	3.4	1.65

Laufmann's Molybdate Figure.—In this method the tannin solution should contain 4 grm. per 250 c.c. of water. 10 c.c. of the filtered solution is

[1] *Collegium*, 1914, 525, 2.
[2] *Leath. Tr. Review*, 1912, 901.
[3] *J. A. L. C. A.*, 1914, 9, 41.
J. A. L. C. A., 1913, 8, 143.
J. A. L. C. A., 1914, 9, 33.

mixed with 10 c.c. of the reagent which is composed of equal volumes of a 10% solution of ammonium molybdate and a 15% solution of ammonium chloride. The precipitate formed is filtered off and 10 c.c. of the filtrate from which the tannin has been precipitated is evaporated to dryness (A). The precipitate on the filter paper is dissolved off with hot water, and the remaining filtrate added to the same, washing the pipette and beaker containing the same with water which is also added to the same solution. The whole is then evaporated to dryness (B). Both the residues are dried until constant weight is obtained which takes from 8–10 hours. The weight of the precipitate obtained is therefore B–A. The figure is expressed on the per cent. of the tannin obtained. For this the total solubles in 10 c.c. of the above solution is estimated in the usual manner, and the amount of tannin in the original solution (C) calculated from the non-tan figure. The molybdate figure is then obtained as follows:

$$\frac{(B-A)100}{C} = \text{Mo. figure.}$$

Myrabolans.—The bloom has been identified by Paessler and Hoffman[1] as crystalline in character and containing chebulinic acid ($C_{28}H_{22}O_{19}$ or $C_{28}H_{24}O_{19}$). Its specific rotatory power is $[\alpha]_D = 64.41°$ for a 3% solution (water-alcohol). It is thought that the acid is present in the fruit as a glucoside, and that this is subsequently split by enzymic hydrolysis.

Mangrove.—The solid extract contains 12% water, 68.5% tannin and 17.3% non-tans. Unless the extract is to be used as a dye it is decolourised by blood albumin. The Queensland variety contains 39% tannin[2]. The Madagascar variety 43–44%; Celebes, 45–48%; E. Africa, 38–42%. As a result of a great number of analyses, made under the direction of J. Paessler, of German East African samples the following results were obtained for the tannin present:

	Lowest	Highest	Mean
Rhizophora....................	29.3%	40.8%	36.5%
Bruguiera....................	24.8	42.3	35.8
Ceriops....................	24.2	32.2	25.8
Xylocarpus....................	26.7	32.5	29.8

The average quantity of water present was 14%. The absence of sodium chloride in an extract precludes the presence of mangrove (Lauffmann) although its presence may be due to other causes. A new source of mangrove extract is said to be the black mangrove (*Avicennia nitida*) of the Bahama Islands.

Cutch.—This material is not the original cutch made in India and used in dyeing, but it is now made from a species of Borneo mangrove, the so-called

[1] *Ledertechn. Rundschau*, 1913, **5**, 129.
[2] *Coombs and Russell, J. Soc. Chem. Ind.*, 1912, **31**, 212.

tengah bark or bastard mangrove.   It gives a light golden colour to leather.
Its analysis is as follows:

```
Moisture........................................    19.4 %
Total Solids....................................    80.16
Soluble solids..................................    79.10
Insoluble.......................................     1.06
Non-tannins.....................................    20.94
Tannin..........................................    58.16    (Bachus).
```

Mallet Bark (*Eucalyptus occidentalis*).—This bark has been on the market
for 8–9 years whence it came from Australia.   Only the flesh of the bark is
used, the *ross* being of little value.   The tannin is easily soluble, 95% of it
dissolving in cold water.   The temperature of extraction must not exceed
60–70°.   An analysis gives 38% tannin; non-tans 11% which include 1.4%
dextrose and 0.8% cane sugar.   The solutions keep well and give a satis-
factory colour to leather.

Chestnut-oak Bark.—Differences in the percentage of tannin as also in
the non-tans may occur in extracts prepared from new or stored bark.   New
bark produces more liquor of a poorer quality.   The difference consists not
in the amount of tannin present in the stored bark but in the greater solu-
bility of the tannins present.   The extract from the old bark being better
quality.[1]

A comparison of thirty-three specimens of chestnut wood (14.5% H_2O)
gave the following great variations on analysis:

```
Tannin...............................  8.1 to 17.2 % (filter method)
Tannin...............................  7.1 to 15.8   (shake method)
Non-tans to 100 pts. tans............   10 to 47     (filter)
Non-tans to 100 pts. tans............   20 to 65     (shake)
```

In cases where low tannin results were obtained with high non-tans the sam-
ples of bark were obtained from young wood.   The results obtained from a
representative wood and an extract showed the following great differences:

	Chestnut wood		Chestnut extract	
	(filter)	(shake)	(filter)	(shake)
Tannin..........................	10.0	9.2	30.0	28.5
Non-tans	1.7	2.5	9.5	11.0
Non-tans to 100 tans............	17.0	27.0	32.0	39.0

The analyst must remember that the ratio of non-tans to 100 tannin will
also vary greatly with the method of extraction of the wood bark or fruit.
It is generally higher when extracted commercially than when extracted in the
laboratory.

Differences are brought about by variations in pressure during extraction.
Thus extracting for 1 hour the following results have been obtained with
chestnut wood.[2]

[1] Smoot, *J. A. L. C. A.*, 1913, **8**, 1071.
[2] Paessler, *Ledertech. Rundschau.*, 1912, 361.

	1 atmos.	3 atmos.	4 atmos.	5 atmos.
Tannin (shake method)..................	11.2	11.2	10.9	9.6
Non-tans.........................	3.4	4.3	7.1	11.5
Tans (100) to non-tans..................	30.0	38.0	65.0	120.0

The effect of extraction under pressure in an autoclave is also seen in the following figures:

	Chestnut		Oak	
	Open	Pressure	Open	Pressure
Tannin.....................................	32.0%	29.8%	31.3%	26.5%
Non-tans..................................	7.3	12.0	8.2	14.0
Insolubles.................................	0.0	0.0	0.0	0.5
Water.....................................	60.7	28.5	60.5	59.0

Both these extracts stood at 25°Bé. It is said that to prevent fermentation sodium fluoride may be present up to 0.3% of the weight of extract.[1]

The sugars, both dextrose and cane sugar, also increase with pressure very rapidly.

Chestnut extracts are noted for their low ash which varies between 0.2 and 0.9%.

Oak Extract.—Results obtained with typical samples of oak extract containing 60% water were as follows (Paessler):·

	Filter method	Shake method
Tannin...	25.5–27.5%	23.0–25.0%
Non-tans...	13.5–11.5	16.0–14.0
Dextrose...		4.05
Cane sugar.......................................		2.5–1.5
100 pts. tans: Non-tans...........................	42–35	56–66

For comparison with the above results oak wood itself gave the following values:

            Tannin (filter)..9.0–13.2%
            Non-tans. (filter)...4.9– 6.5
            Ratio 100 tans. to non-tans.................................35–54

The finished extract contains 0.6–1.3% acetic acid according to Jedlicka.[2]

Oak Extract[3] owing to its high sugar content is specially liable to fermentation. It is a mixture of the pyrogallol and catechol tannins.

Emory oak occurs in the semi-arid regions of the south-western part of the United States. The bark gives 16.1% total solids; 14% solubles; 6.6% nontans.; 7.4% tans.; 2.1% reds. On the other hand the wood itself gives total

[1] Thuau, Le Cuir, 1913, p. 595.
[2] Collegium, 1913, 514, 33.
[3] U. J. Thuau, Le Cuir, 1913, p. 595.

solids 8.3%; solubles 7.5%; non-tans. 4.4%; and tans 3.1%. The colour of the tannin is rather dark.

Quebracho.—A normal liquid extract of this wood may contain 34.5% tannin, 3.5% non-tans.; water 60%, dextrose 0.3%, cane sugar 0.2%; and ash 1.0%. An extract showing high non-tans. (6.5%) also gave a high ash (3.0%). In a special case an extract giving 28.7% tans., 10.7 non-tans., 12.2 dextrose and 2.9% ash was regarded with suspicion, and a qualitative test indicated the presence of myrabolans (Paessler). Schell[1] has suggested a special test to detect mangrove in quebracho extract.

The ratio of insoluble tans. to soluble ones is given by W. Moeller[2] as 1 : 10 at analysis strength. It increases at further concentration to 2:3 at 8°Bé. but on continuing the concentration to 20°Bé., solution is complete.

The adulteration of quebracho extract by mangrove may, according to van Gijn and van der Waerden,[3] be detected by estimating the pentoses and pentosans present in the extract by the usual Tollens-Kröber method of determining furfural. Quebracho extract is almost free from pentosans and pentoses, while mangrove contains fair quantities of these substances. Details of the method of estimation may be found in the original communication. W. Moller[4] criticises this method.

Lauffmann on the other hand proposes to precipitate the tannin by his ammonium molybdate method. With untreated quebracho extract the Mo-figure varies from 28 to 37, but unfortunately in sulphited quebracho this varies from 5 to 37. Mangrove extract gives a figure between 120-130 and sulphited mangrove extract 111. Stiasny[5] has confirmed that mixtures of these two extracts act satisfactorily with this test, but it must be noted that if a Mo-figure of 30 be taken as a standard for quebracho extract, and anything above this be regarded as due to added mangrove extract, an error equal to 20% of mangrove may be looked for if the quebracho extract is a sulphited extract giving an actual figure of 5. In making this test care must be taken to see that no pyrogallol-tans. (formaldehyde test) or sulphite cellulose (aniline test) be present. Stiasny and Wilkinson have shown that an additional sulphite process in the laboratory to equalise the original quebracho extracts to a common sulphite basis is unsatisfactory. They consider that further work will be necessary before this test can be considered as authoritative. G. A. Kerr proposes to identify the addition of mangrove by a phloroglucinol test: 100 c.c. of hydrochloric acid (12%) are placed in a 250 c.c. Erlenmeyer flask, and 2 grm. of the tannin material added in the dry state (or an equivalent amount of extract) with few pieces of pumice stone to prevent bumping. The solution is distilled through an ordinary glass condenser at the rate of 30 c.c. every 10 minutes, 30 c.c. of hydrochloric acid being added through a thistle head tube as each 30 c.c.

[1] *J. A. L. C. A.*, 1912, 7, 564.
[2] *Leadertechn. Rundschau*, 1913, 8, 258.
[3] *J. A. L. C. A.*, 1914, 9, 109.
[4] *Collegium*, 1914, 531, 85.
[5] *Collegium*, 1914, 526, 77.

distil over. Distillation is carried on until 300 c.c. have passed over. The distillate is tested by placing 100 c.c. in a glass beaker (2 in. diam.) and adding 8 c.c. of a solution of phloroglucinol (made by dissolving 0.25 grm. in 25 c.c. of 12% hydrochloric acid). The solution is stirred for a few moments until the colour reaches its maximum (within 5 minutes). This remains permanent for some time. With pure quebracho the colour is first a brilliant yellow, gradually becoming a bright green and finally a bluish tint as the precipitate forms, which is a dense black. With mangrove the first colour is orange, developing to a deep orange and the precipitate is buff coloured instead of black. In mixtures of quebracho and mangrove it is claimed that even so low an addition of the latter as 5% will change the colour to olive. At 50% the green colour is dominated by the orange of the mangrove. Under a low power, the precipitates may be seen side by side when small additions of mangrove are present.[1]

Turwar Bark (*Cassia Auriculata*).—The maximum tannin is extracted from this bark only at 85°–90°. No dextrose could be detected in the extract. The lead acetate precipitate has a peculiar chocolate colour.

White Tan (*Cæsalpinia Digyna*).—This is said to contain 35% of tannin and to produce a leather equal to sumac in colour. The tannin-bearing pods grow well in Burmah. The pod cases contain the tannin (Faust) to the extent of 40.07%; the non-tans. being 18.68%; insolubles 3.47% and moisture 7%.

Sumac.—In the detection of sumac in the state of leaf the microscopical examination of the leaf cuticle is of great value. This was originally pointed out by Lamb.[2] When the sumac leaves are adulterated they are generally supplied in a half or wholly ground condition, and therefore microscopical examination is necessary to disclose structure; a 1 in. objective is sufficient to detect the difference between sumac and adulterant material after the treatment recommended by Lamb. The most common adulterant is the leaves of *Pistacia Lentiscus*, which grow abundantly in Cyprus. It has even been stated that some 10,000 tons of this material are used annually to adulterate sumac. The *Tamarix Africana* is also used for this purpose. Lamb found that not more than 10% of a number of samples were unadulterated. His method of procedure is as follows: 1–2 grm. of the sample are placed in a large boiling tube and covered with nitric acid (1:1). The mixture is well shaken to thoroughly wet the sumac and the tube gently heated over a small Bunsen flame until nitrous fumes are evolved. The tube is then left to stand for 15–30 minutes. At the end of this period the tube is again heated until the solution becomes quite clear. An excess of water is then added and the mixture filtered through a close textured filter paper and the residue washed with distilled water. A small hole is then made in the filter paper and the residue washed through

[1] *J. A. L. C. A.*, 1914. 9, 27.
[2] *J. Soc. D. and Col.*, 1890.

into a basin with distilled water. A few drops of a solution of dyestuff added and the mixture gently warmed for a few minutes until the small particles are coloured but not so deeply as to lose their transparency. Bismarck brown, Safranine, and Methylene Blue are suitable for the purpose. After getting rid of the surplus dye solution the particles are filtered off as before through paper, washed with a little water, and a hole once more made in the filter paper and the residue washed into a clean porcelain basin. A number of the dyed particles are transferred to a microscope slide and a cover-glass placed over them. A reference to the original paper will show the considerable difference observed between the true sumac and the adulterants. · The cellular structure of the adulterants is quite distinctive; the stomata afford a valuable means of identification. The treatment with nitric acid if prolonged dissolves the cuticle of sumac and leaves nothing more than what has been described as a "wreck," while the adulterants are not acted on in this manner. (See also *J. Soc. D. and Col.*, 1904. **20,** 265.)

Tannin Extracts.—It is not possible to consider in detail the manufacture of these extracts, but the following results of analysis from different authoritative sources will serve to indicate their nature (official method of analysis):

Extract	Total solids	Insolubles	Tannin	Non-tans.
Quebracho solid, unclarified.............	88.04	7.49	74.01	6.55
Do. liquid, pure......................	....	....	30.07	5.92
Chestnut extract, liquid...............	42.95	0.35	28.45	14.15
Do................................	....	....	27.6	11.6
Oak bark liquid......................	45.8	1.47	25.3	19.03
Hemlock liquid......................	50.08	3.43	25.6	20.96
Sulphite-cellulose liquid...............	49.97	0.12	26.76	22.93

Non-tans.—The ratio of tans. (100) to non-tans. varies greatly according to the nature of the extract, as will be seen in the following table (Paessler). This fact may be utilised to determine the origin of an extract. At the same time the great differences which may be met with in the same extract prepared in different ways must always be remembered:

Extract	Tans.	Non-tans.	
		Filter method	Shake method
Pine bark.........................	100	69	93
Oak bark.........................	Do.	60	74
Sumac............................	Do.	60	74
Myrabolans........................	Do.	35	49
Divi-divi..........................	Do.	41	55
Mimosa bark.......................	Do.	32	39
Valonia...........................	Do.	37	49
Mangrove.........................	Do.	25	30
Quebracho wood....................	Do.	8	14

That the ratio of tans. to non-tans. may vary greatly in the same kind of extract is seen from the following figures (Paessler). The three extracts were chestnut extracts (25°Bé.).

Sample	Tans.		Non-tans.		Ratio tans./non-tans.	
	Filter method	Shake method	Filter method	Shake method	Filter method	Shake method
1	34.1	32.2	5.1	7.0	15	22
2	10.0	28.5	10.0	11.5	33	40
3	26.5	25.6	12.7	13.6	48	53

Detection and Differentiation of Vegetable Tannins.—The detection of adulteration of tannins by cheaper tannins is a matter of great difficulty to the general analyst, or even to the specialist.

Lead Acetate Test.—To 5 c.c. of tannin solution (analytical strength) 5 c.c. of 10% lead acetate solution are added and a portion of the clear filtrate is mixed with an excess of sodium hydroxide (10%). Mangrove, mimosa, oakwood, chestnut, myrabolans, valonia, divi-divi, algarobilla and gallic acid give colourless solutions. Quebracho and ulmo give a slight but distinct colouration, whilst *Pistacia lentiscus* and sumac give a decided yellow and wood pulp a deep yellow colouration (Stiasny).

Acetic Acid Lead Acetate Test.—This test has been applied quantitatively to the separation of certain tannins (Dreaper, Vol. 5, p. 70). In the following test (Stiasny) 5 c.c. of tannin solution (analytical strength) are mixed with 10 c.c. of acetic acid (10%) and 5 c.c. of lead acetate (10%) and the formation of the precipitate of lead tannate observed.

Catechol tannins (quebracho, mangrove, mimosa, ulmo, and gambier) give no precipitate.

Pyrogallol tannins (chestnut, oakwood, myrabolans, sumac, valonia, divi-divi, algarobilla, and gallo-tannic acid) give precipitates. (Note: *Pistacia lentiscus* behaves like a pyrogallol tannin in this test.) The precipitate is filtered off and the clear solution tested with a few drops of ferric alum solution (1%). Quebracho, mangrove, ulmo and gambier give a green colour, whilst mimosa, myrabolans, sumac, divi-divi, algarobilla and gallo-tannic acid give a deep bluish violet. Chestnut gives a very faint violet, whilst oakwood and valonia remain colourless. By this test it is claimed that an addition of 25% of chestnut to an oakwood extract can be detected.

Formaldehyde Test.—In its recent form this test is as follows: To 50 c.c. of tannin solution (analytical strength) 5 c.c. of concentrated hydrochloric acid are added and then 10 c.c. of formaldehyde (40%). The mixture is boiled in a reflux condenser for 30 minutes, cooled to the ordinary temperature and filtered. The appearance of the precipitate whilst boiling is noted and the filtrate used for the following test: To 10 c.c. of the filtrate 1 c.c. of ferric alum solution (1%) is added and about 5 grm. of solid sodium acetate, without shaking. Observe if a blue (or violet) colour appears in the lower layer. Catechol tannins are entirely precipitated by this reagent, the filtrate giving no indication with the iron salt. A portion of the pyrogallol tannins may be precipitated under this treatment, but all of them respond to the test with the iron salt. In practice this test may be

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used to determine admixture of pyrogallol tannins with catechol tannins. Certain sulphated quebracho extracts (catechol) are not entirely precipitated by this reagent (Schell). After 30 minutes' boiling in the prescribed manner these special extracts are not entirely precipitated. In this case, however, and even if the filtrate be strongly coloured, it will give no distinct indication, or at least no blue colouration after the addition of the ferric alum solution and sodium acetate (Stiasny). This treatment with formaldehyde does not give a quantitative separation, as a considerable proportion of the pyrogallol tannins are co-precipitated with the catechol tannins. Small[1] has suggested a modified process which Stiasny thinks may give a quantitative separation.
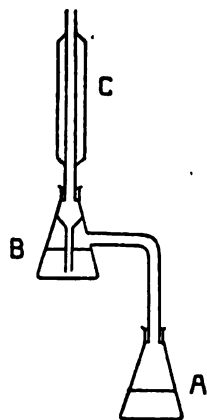
Solubility Test.—This test as modified by Stiasny and his pupils[2] is as follows: In the original test[3] 25 c.c. of the tannin solution were shaken out with 25 c.c. ethyl acetate until the latter remains colourless. The dry residue of the aqueous layer was compared with the total solubles of a corresponding volume of the original aqueous solution. Owing to the fact that ethyl acetate is partially soluble in water, Stiasny now suggests that it is advisable to pass a current of air for about 10 minutes through the extracted aqueous solution before taking, say, 20 c.c. for evaporation. The difference stated in percentages gives the solubility in ethyl acetate. It has been suggested that the ethyl acetate shall be replaced by amyl acetate owing to the greater insolubility of the latter in water. The test is further improved by utilising the apparatus suggested by Stiasny (Fig. 14). 50 c.c. of the amyl acetate (or ethyl acetate) are placed in flask A and distilled, by means of a water-bath in the case of ethyl acetate or an oil-bath in the case of amyl acetate, into the flask B, in such a way that the solution condensed in C, drops into the funnel and is forced to pass through the tannin solution in B before it runs back into A, through the side tube. With amyl acetate the flask B is placed in a basin with a continuous stream of cold water passing through it. The alcohol solubility figure is obtained as described in *Collegium*, 1911, 107. 10 c.c. of the solution of tannin (analytical strength) are placed in a 100 c.c. flask which is filled to the mark with absolute alcohol. 50 c.c. of the filtrate are evaporated to dryness and the weight of the dry residue compared with the total solids (solubles) of a corresponding volume of the original liquor. Reference must be made to the original communication for the figures obtained. With quebracho extract (and wood) the amyl acetate figures show 70–80% extraction by this reagent. Unfortunately sulphiting this extract greatly

FIG. 14.

[1] *J. A. L. C. A.*, 1911, **6,** 107.
[2] *J. A. L. C. A.*, 1912, **7,** 554.
[3] *Collegium*, 1911, 325.

reduced this figure. Complete sulphiting practically renders the tan. insoluble in amyl acetate. This greatly interferes with the validity of this test in the case of an unknown extract. Mangrove also seems to give varying results, the average extraction is about 8–10% but in one case gave 42%. The average extraction with chestnut is 10. Oakwood extract gives figures between 0–12%. It will be seen that considerable experience is necessary before this solubility test can be utilised, and then the results must be taken in conjunction with other tests. The sulphiting process practised by extract manufactures greatly interferes with the results obtained.

Bennet's Test.—2–3 c.c. of the tannin solution are mixed with 1 or 2 drops of a 10% solution of sodium bisulphite and an equal amount of potassium chromate. All catechol tannins are said to give a greenish colour. On the other hand, some pyrogallol tannins (myrabolans, sumac, and gallotannic acid), give a blood-red colouration which rapidly fades to brown. Other pyrogallol tannins (valonia, chestnut and oakwood extracts) give a deep violet which is fairly stable. The test is claimed to be useful for single tannins, or for subdividing the pyrogallol tannins into two subgroups. It is, however, of little value in the frequently occurring case where chestnut or oakwood is adulterated with myrabolans.

Konstein's Test.—The tannin is precipitated by *alcutin* (an albuminose obtained from Dr. Meyersburg, Sumper Gasse 37, Vienna) the solution filtered and a little strong ammonia added to the filtrate, which is then boiled and the change of colour observed.[1]

Hoppenstedt Test.—This is said to distinguish mangrove tannin from other extracts but can only be utilised when the mangrove extract is pure. To 25 c.c. of tannin solution (analytical strength) 25 c.c. of quinine hydrochloride (1%) solution are added and the precipitate filtered off; 1 c.c. of glacial acetic acid is added to 5 c.c. of the filtrate in a test-tube and then 5 c.c. of ethyl acetate. The liquid is mixed thoroughly by shaking and allowed to separate into layers. With mangrove the lower layer is coloured a strong yellow brown; with the other tannins the layer is colourless.

Dieterich Test.—This serves to detect the presence of gambier. Add 5 c.c. of alcohol to 5 c.c. of the tannin solution. After shaking add 1 c.c. of 1% potassium hydroxide solution. Then add 10 c.c. of petroleum spirit, mix, and allow the layers to separate. With gambier the upper layer exhibits a strong green fluorescence. It is claimed that this test will apply to mixtures (Hoppenstedt).

Hoppenstedt Test for Hemlock.—To 50 c.c. of the tannin solution add 10 grm. calcium chloride (anhydrous), agitate until dissolved, cool, and filter. 5 c.c. of the filtrate are placed in test-tube, 1 c.c. of glacial acetic acid added, then 5 c.c. of amyl acetate; shake and allow layers to separate. With hemlock the upper layer is coloured a strong yellow

[1] *Collegium*, 1912, 153; see also *J. A. L. C. A.* 1912, 7, 565.

brown; with other tannins it is colourless. This is claimed to be efficient in identifying hemlock in mixtures.[1]

Eitner-Philip Sulphide Test.—This test as now given by Procter is as follows.[2] Add 2–3 drops of strong sulphuric acid to 25 c.c. of a strong tannin solution (2.5%) in a flask and boil for 1–2 minutes; cool, add about 5 grm. of salt, shake and allow the mixture to stand for 5–10 minutes and filter off the precipitate. In a test-tube add 10–15 drops of ammonium sulphide solution to about 15 c.c. of water and then 2–3 c.c. of the above filtrate. All pyrogallol tannins give a copious precipitate of varying colour whilst most catechol tannins give no precipitate even after standing over night. Procter (*ibid.*) states that mimosa and malet behave like pyrogallol-tans toward this test and that they can therefore be easily detected even in mixtures with other catechol tans. It must be remembered, however, that other tannins (pine, catechu, and gambier) are also precipitated by ammonium sulphide solution.[3]

Assuming that the total tannin as returned by the official method really consists of tannic acid and certain neutral substances R. Vanicek[4] proposes to estimate the acid in the original solution by titrating with $N/10$ sodium hydroxide, using phenolphthalein by the "spotting out" method. A portion of the original solution is then detannised with gelatin and the titration repeated. The difference is calculated as tannic acid, using a predetermined factor for each different tannin under examination. Stiasny considers that the values of the available tests stand in the following order:

Solubility test
Formaldehyde test
Acetic acid-lead acetate test
Aniline test
Bromine test

and that these must be taken in conjunction with the amount of tans and non-tans and their ratio and also the Löwenthal figure. There is no doubt that the latter process is coming into greater repute again and in this respect the figures given in Vol. 5 serve a useful purpose. The advent of sulphite-cellulose extracts and the fact that their active constituent is returned by the official method as tannin is one of the chief causes of the return to this process.

In extreme cases the refractometer method may be of some value and further work has been done in this direction by Kubelka.[5]

As has been before pointed out, when the tannin material is to be used for other purposes than that of tanning skins, the problem presented to the analyst is of a different order and this must always be remembered when deciding upon the methods to be used to estimate the tannin present. Thus in dyeing silk, the weight-giving properties of the extract in combination with its colour, alone and in the presence of mordants, are of special significance.

[1] *J. A. L. C. A.*, 1912, 7, 172.
[2] *Leather Chemist's Pocket Book*, 1912.
[3] *J. A. L. C. A.*, 1909, 4, 249.
[4] *Zeit. angew. Chem.*, 1913, 26, 68.
[5] *Collegium*, 1914, 527, 151.

It has been shown by Knowles[1] that the results obtained by the official
method (A. L. T. C.) do not correspond with the results obtained on cotton
with subsequent saddening with ferric sulphate, nor does the percentage of
iron taken up agree with the tannin present as determined by the official
method. Again in the dyeing of silk, some tannin-extracts although return-
ing a high percentage of tannin, are quite unsuitable for certain purposes.

The chief tests for the differentiation of tannins have been conveniently
collected by Stiasny in the following tables.[2] An interesting use is made of
the formaldehyde test at both 15 and 20 minutes.

Group I.—Complete precipitate: the filtrate gives neither gelatin test nor
iron test.

Tests for confirmation; bromine test (precipitate) and acetic acid-lead
acetate test (no precipitate).

Group II.—No precipitation during 15 minutes' boiling.

Test for confirmation: bromine test (no precipitate); ammonium sulphide
test (precipitate).

Group III.—Considerable precipitate during boiling, but distinct iron
test of the filtrate.

To Group I belong: quebracho, mangrove, ulmo, gambier, pinebark,
hemlock, mimosa, malet.

To Group II belong: oak-wood, chestnut-wood, valonia, myrabolans.

To Group III belong: oakbark, Pistacia lentiscus, sumac, divi-divi,
algarobilla, teri, bablah, galls.

Having found to which group the tannin belongs, the following tests are
made in each group:

Further testing of Group I.—The ammonium-sulphide test allows a sub-
division, in so far as no precipitate is obtained with quebracho, mangrove,
ulmo, gambier, pinebark, hemlock (Group Ia) while a precipitate is shown
by minosa and malet (Group Ib).

Group Ia is also characterized by the green colouration produced with
iron alum.

Group Ib gives a bluish violet with iron alum.

The further way of identifying the tannin in Ia or Ib, demands the carry-
ing out of all the tests mentioned in previous papers and summarized in Table
II. This table also contains the gallic acid value of 1 grm. of the tannin
and the proportion of tans to non-tans in the tanning material. The Mo
figures found by Lauffmann are also given in the table.

Further testing of Group II.—The acetic acid-lead acetate test allows
a subdivision, as no colouration is given on adding iron alum to the filtrate
of the lead precipitation, in the case of oak-wood and valonia (Group IIa);
while a more or less distinct violet colouration is obtained with myrabolans
and chestnut (Group IIb).

[1] *J. Soc. D. and Col.*, 1912, 28, 174.
[2] *J. A. L. C. A.*, 1914, 9, 22.

TABLE I.

50 c.c. tannin solution (0.4%) boiled with 25 c.c. of the formaldehyde-HCl mixture for 30 minutes, thoroughly cooled and filtered.

Complete precipitate: Filtrate+iron alum+sodium acetate: no violet colouration.	No precipitate after 15 minutes boiling.	Considerable precipitate after 15 minutes boiling; deep violet colouration of the filtrate+ iron alum+sodium acetate.
GROUP I	**GROUP II**	**GROUP III**
Confirming tests: +bromine: precipitate. +acetic acid+lead acetate: no precipitate.	Confirming tests: +bromine: no precipitate. Ammonium-sulphide test: precipitate.	
25 c.c. tannin solution (2.5%)+ammonium-sulphide test.	5 c.c. tannin solution (0.4%)+acetic + acid lead-acetate test. The filtrate of the precipitate gives+iron alum.	5 c.c. tannin solution. (0.4%)+bromine test.
No precipitate. Precipitate.	No colouration. Violet colouration.	Precipitate. No precipitate.
GROUP Ia **GROUP Ib** Confirming tests: + iron alum.	**GROUP IIa** **GROUP IIb** Oakwood Chestnut Valonia Myrabolans	**GROUP IIIa** **GROUP IIIb** Oakbark Súmac Pistacia Divi
green bluish violet Quebracho Mimosa Mangrove Malet Ulmo Gambier Pine-bark Hemlock		Algarobilla Galls Bablah Teri

The sugar content of tannin extracts plays an important part in actual tanning. Apart from this the variations observed in the amounts present may also indicate the nature of the extract, and therefore be useful to the analyst.

The following table due to Paessler[1] indicates the differences observed when using pure extracts; the shake method was adopted for the separation of the tannin.

Material	Non-tans.	Monosaccharides	Disaccharides	Total sugars
Pine bark...............	93	33	14	47
Oak bark................	74	30	0	30
Sumac..................	74	17	2	19
Myrabolans..............	49	18	0	18
Divi-divi...............	55	11	4	15
Mimosa.................	39	6	8.5	14.5
Valonia.................	49	12	0	12
Trillo..................	44	7.5	1.5	9
Mangrove...............	30	2	1	3
Quebracho wood.........	14	1	1	2

Tannin Substitutes.

A good deal of attention has been paid to these materials both as regards sulphite-cellulose products ("pseudo-tannins") and the more recently introduced synthetic tannins.

Sulphite Cellulose Products (pseudo-tannins).—The use of these products and their admixture with tannin extracts has drawn attention to the disturbing action of such substances on the analysis of tannin materials.

[1] *Collegium*, 1913, 516, 157.

TABLE II

| | Formaldehyde test | | Bromine test | Ammonium-sulphide test | Lead-acetate test. Filtrate+NaOH | Acetic acid+lead acetate test | | Ethyl-acetate figure | Alcohol figure | Gallic acid value of 1 gm. tannin. (See Collegium, 1909, p. 191) | Tans/Non-tans | Molybdenum figure (Lauffman) |
	During 15 min. boiling	Filtrate+iron alum+sodium acetate					Filtrate+iron alum					
Quebracho	pp.	No colouration	pp.	No pp.	Yellowish	No pp.	Green	70-80	0-5	0.59	8.0-10.0	25-40
Sulphited-quebracho	pp.	No colouration	pp.	No pp.	Yellowish	No pp. (but PbSO₄)	Green	0-70	0-5	0.59	Depends on the method of sulphiting	0-20
Mangrove	pp.	No colouration	pp.	No pp.	Colourless	No pp.	Green	0-5	0-5	0.68	2.5- 4.0	100-135
Ulmo	pp.	No colouration	pp.	No pp.	Yellowish	No pp.	Green	70-80	0-5		8.0-10.0	42
Gambier	pp.	No colouration	pp.	No pp.	Colourless	No pp.	Green	50-65	5-10	0.56	1.2- 1.5	0-13
Mimosa	pp.	No colouration	pp.	pp.	Colourless	No pp.	Deep bluish violet	30-40	0-5	0.53	2.0- 3.0	110-130
Oakbark	pp.	Violet	pp.	pp. (after standing over night)	Colourless	pp.		12	17		1.0- 1.5	135
Hemlock	pp.	No colouration	pp.	pp.	Yellowish	No pp.	Green	18	9		1.0- 2.0	65-85
Pistacia	pp.	Deep bluish violet	p.	pp.	Yellow	pp.	Green & violet	3	29			
Chestnut	No pp.	Deep bluish violet	No pp.	pp.	Colourless	pp.	Very faint violet	0-16	10-20	0.56-0.66	2.0- 3.5	180-225
Oakwood	No pp.	Deep bluish violet	No pp.	pp.	Colourless	pp.	Colourless	0-12	20-30	0.5 -0.56	1.0- 2.0	175-210
Myrabolans	pp.	Deep bluish violet	No pp.	pp.	Colourless	pp.	Violet	30-50	0-15	0.55-0.60	1.5- 2.5	80-140
Sumac	pp.	Deep bluish violet	No pp.	pp.	Yellow	pp.	Violet	40-60	5-20	0.65-0.69	1.5- 1.8	125-155
Valonia	Turbid	Deep bluish violet	No pp.	pp.	Colourless	pp.	Colourless	5-15	20-40	0.55-0.63	2.0- 3.0	222
Divi-divi	Turbid	Deep bluish violet	No pp.	pp.	Colourless	pp.	Violet	30-50	0-10		?	
Algarobilla	Turbid	Deep bluish violet	No pp.	pp.	Colourless	pp.	Violet	50-60	0-5		?	
Wood pulp	No pp.	No colouration	No pp.	Not characteristic	Deep yellow	No pp.	Colourless	0-5	30-70	0.09-0.14	0.75	

The fact that sulphite extracts contain substances which are absorbed by hide powder (and are therefore returned as tannin material) is a disturbing one to the analyst who is bound to use the official methods of the European and American Associations.

Sulphite cellulose extracts give precipitates with gelatin and the alkaloids, but fail to give the characteristic indications with other reagents used to identify tannins. As the cellulose extracts are almost always used in conjunction with normal tannin extracts and frequently mixed with them as adulterants, too much attention must not be given to the absence of certain reactions when the pure sulphite extracts are tested. They give no colour indications in the pure state with ferric salts, no red colouration with ammonia and ferrocyanide, nor any precipitate with bromine or with formaldehyde and hydrochloric acid. No precipitate is formed with tartar emetic and salt. The absence of these reactions in conjunction with a precipitate with gelatin and salt and the *apparent tannin content obtained by the hide powder process* will indicate the absence of any real tannin material and the presence of sulphite cellulose.

When treated with solvents the sulphite extracts also give certain characteristic results. Extraction with alcohol, acetone, or acetic ether, dissolves out some soluble substance, but these do not give the characteristic reactions of tannins.[1] Ethyl alcohol dissolves out a resinous substance insoluble in water after removing the solvent by evaporation. As previously mentioned the permanganate process of analysis gives figures "entirely outside the range of the tannins." For example a sulphite-cellulose extract giving 25% tannin by the official hide powder process only gave 5% tannin by the permanganate process. Both these processes therefore give a tannin result when no tannin is present. The analyst who is not constantly examining tannin materials should be specially careful to make sure that these products are not present in any material he is examining.

That sulphite-cellulose is undoubtedly absorbed by skins and gives a leather of sorts, and that the substances so absorbed are fairly proof against the subsequent dissolving action of water is certain. The relative weight-giving properties of these false extracts and certain tannin materials are as follows:[2]

Sulphite cellulose	100.0
Gambier	110.5
Sumac	115.1
Valonia	118.9
Oak bark	120.6
Chestnut	124.5
Quebracho	125.7

The relative cost per weight is given by the same authority to be

Sulphite cellulose	0.000357
Gambier	0.001267
Sumac	0.001130
Valonia	0.001093
Oak bark	0.000829
Chestnut	0.000498

[1] *J. A. L. C. A.*, 1913, **8**, 67.
[2] Wisdon, *J. A. L. C. A.*, 1913, **8**, 233.

Levi and Orthmann[1] claim that their "reagent 33" does not give positive figures for sulphite cellulose and that therefore the process when taken in connection with the official hide powder process indicates the amount of sulphite cellulose present in an extract. Unfortunately the actual relationship between the hide powder figures and those given by reagent 33 has not been so definitely established as the authors seem to suggest. The Dreaper copper process also does not give results with sulphite cellulose extracts. (See Vol. V, p. 70.)

Eitner mentions[2] that the addition of these false extracts to a true extract not only cheapens the latter but improves the appearance of thick inferior quebracho extract. It has also been used as a substitute for chestnut when mixed with an addition of myrabolans, quebracho or mangrove. The ratio of the tans to non-tans by the hide powder process indicates by its variation the presence of the sulphite extracts The sulphite extracts show a ratio of 1/0.82, whilst true vegetable tannins give figures ranging from 1/10 (quebracho) to 1/2.3 (oakwood). It has been stated that its addition to quebracho extract facilitates diffusion into the skin.

In Procter's test (previously mentioned) for the detection of these false extracts, in which to 5 c.c. of a tannin solution, 0.5 c.c. of aniline is added and after shaking 2 c.c. of concentrated hydrochloric acid, it must be noted that a slight cloudiness does not necessarily suggest adulteration. The ordinary precipitate given by sulphite extract, as compared with a clear solution with a true tannin, is of a definite nature; it is copious and after a time rises to the surface of the liquor. Certain difficulties which have arisen with this test as pointed out by Monnet[3] have been traced by Jedlicka[4] to certain pure Slavonian extracts which give a cloudiness "but that any extract may be certified as free from cellulose extracts if it does not give a flocculent precipitate after 2 hours standing." More recently the A. L. T. C. has through a committee[5] obtained evidence as to the reliability of this test when the above remarks are taken into consideration. An addition of 5% of sulphite cellulose was easily observed and even 2–3% could be detected in some cases.

Synthetic Tans.—The bodies are condensation products obtained by acting on phenols with formaldehyde.

Neradol D.—This artificial product which is claimed to act as a tanning material and has been put on the market by the Badische Anilin und Soda Fabrik is sent out in the form of a heavy liquid or paste of about 40°Bé. and in appearance resembles a vegetable tannin extract. It is readily soluble in water forming a clear solution of a semi-colloidal nature. According to G. Grasser[6] its reactions in water solution are as follows: methyl orange gives a red colouration; copper sulphate, no reaction; amm. copper sulphate, red-

[1] *J. A. L. C. A.*, 1913, **8**, 41.
[2] *Gerber*. 1913, **39**, 43 and 57.
[3] *Collegium*, 1913, **518**, 224.
[4] *Collegium*, 1913, **518**, 317.
[5] *J. A. L. T. C.*, 1914, **9**, 36.
[6] *J. Amer L. T. Chem.*, 1913, **8**, 404.

brown ppt., soluble in excess to deep brown solution; ammonia, no reaction; ferric chloride deep blue colour; lead acetate, white ppt.; gelatin, flocculent ppt.; aniline hydrochloride, heavy white ppt., basic aniline dye, ppt.; no reaction with bromine water, formaldehyde and hydrochloric acid, zinc acetate, sodium sulphite, lime water or iodine water.

Stiasny suggests the following test to distinguish between Neradol D and sulphite-cellulose: 10 c.c. of a 5% solution of the extract are shaken with 1 or 2 drops of a 1% solution of alum and about 5 grm. of ammonium acetate. On standing for 24 hours Neradol D remains clear while sulphite-cellulose gives a heavy flocculent precipitate. Neradol D is insoluble in the following solvents: ether, carbon disulphide, chloroform, acetone, and amyl alcohol. It is insoluble except its inorganic constituents, in alcohol, glacial acetic acid and acetic ether.

Analysis by the shake method gives the following results:

Water...	34.5%
Tanning substance...	32.5
Soluble non-tannins...	33.0
Insolubles..	0.0
Ash...	17.4

The ash is composed of sodium sulphate.

ERRATA IN VOL. V.

Title pages ii and iii, for "J. F. Hewitt" read "J. T. Hewitt."
Page 37, line 4, for "Silician" read "Sicilian."
Page 44, line 14, for "Proctor" read "Procter."
Page 83, lines 1 and 19, for "phlobathenes" read "phlobaphenes."
Page 103, line 7, for "*Gazetta*" read "*Gazzetta*."
Page 700, in Index, "Phlobathenes" should read "Phlobaphenes".

ANALYSIS OF LEATHER.

By W. P. DREAPER F. I. C.

Since this matter was treated in Vol. V, p. 105, The American Leather Chemists Association has issued the following Official Method for the analysis of leather.

OFFICIAL METHOD FOR LEATHER ANALYSIS.

(1) **Preparation of Sample.**—The sample of leather for analysis shall be reduced to as fine a state of division as practicable, either by cutting or grinding.

(2) **Moisture.**—Dry 10 grm. of leather for 16 hours at a temperature between 95°–100° C.

(3) **Fats.**—Extract 5 to 10 grm. of air-dry leather in a Soxhlet apparatus until free from grease, using petroleum ether boiling below 80° C. Evaporate off the ether and dry to approximately constant weight.

Or, if preferred, extract 30 grm. of leather as described above. In the latter case, the extracted leather, when freed of solvent, may be used for the determination of water-soluble material.

(4) **Ash.**—Incinerate 10 to 15 grm. of leather in a tared dish at a dull red heat until carbon is consumed. If it is difficult to burn off all the carbon, treat the ash with hot water, filter through an ashless filter, ignite filter and residue. Add the filtrate, evaporate to dryness and ignite.

(5) **Water-soluble Material.**—Digest 30 grm. of leather in a percolator over night, then extract with water at 50° C. for 3 hours. The total volume of solution to be 2 litres. Determine total solids and non-tannins according to the Official Method for extract analysis.

(6) **Dextrose.**

Solutions.

Copper Sulphate.—Dissolve 34.639 grm. of $CuSO_4.5H_2O$ in distilled water and dilute to 500 c.c. Filter through asbestos.

Alkaline Tartrate Solution.—Dissolve 173 grm. of Rochelle salt and 80 grm. NaOH in water and dilute to 500 c.c. Allow to stand two days and filter through asbestos.

Normal Lead Acetate Solution.—Prepare a saturated solution of normal lead acetate.

Determination.[1]

Place 200 c.c. of leather extract of analytical strength in a 500 c.c. flask, add 25 c.c. of a saturated solution of normal lead acetate, shake frequently (5–10 minutes), and filter. (The funnels and beakers must be kept covered to prevent evaporation.) Add to the filtrate an excess of solid potassium oxalate. Mix frequently for 15 minutes and filter, returning the filtrate until clear. Pipette 150 c.c. of this filtrate into a 600 c.c. Erlenmeyer flask, add 5 c.c. of concentrated hydrochloric acid and boil under a reflux condenser for 2 hours. Cool, neutralise (place a small piece of litmus paper in the flask) with anhydrous sodium carbonate, transfer to a 200 c.c. graduated flask and make to volume. Filter through a double filter. (The filtrate must be clear.) Determine the dextrose in the solution immediately.

Place 25 c.c. of the copper solution and 25 c.c. of the alkaline tartrate solution in a 400 c.c. beaker. Add 50 c.c. of the clarified and neutralised solution above mentioned and heat to boiling in *exactly* 4 *minutes* and boil for 2 minutes. Filter immediately without diluting, *through asbestos*,[2] wash thoroughly, with hot[2] water, then with alcohol, and finally with ether; dry for half an hour in water oven and weigh as cuprous oxide, determine the amount of dextrose by the use of Munson and Walker's table (see page 415) and report as percentage on leather.

(7) **Nitrogen.**—Gunning modification of the Kjeldahl Method, A. O. A. C. Bulletin, No. 107 (1909). (See Vol. I).

Reagents.

Standard Acid Solutions.—Hydrochloric or sulphuric acid the absolute strength of which has been accurately determined. For ordinary work $N/2$ acid is recommended. For work in determining very small amounts of nitrogen, $N/10$ is recommended. In titrating mineral acid against hydroxide solution use cochineal as indicator.

Standard Alkali Solution.—The strength of this solution relative to the acid must be accurately determined; $N/10$ solution is recommended.

Sulphuric Acid.—The sulphuric acid used should have a sp. gr. 1.84 and be free from nitrates and also from ammonium sulphate.

Sodium Hydroxide Solution.—A saturated solution of sodium hydroxide free from nitrates.

Potassium Sulphate.—This reagent should be pulverised before using.

Indicator.—A solution of cochineal is prepared by digesting and frequently agitating 3 grm. of pulverised cochineal in a mixture of 50 c.c. of

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[1] The rate of heating of the Bunsen burner used should be regulated before sugar determinations are started. This is best done by adjusting the burner so as to bring 25 c.c. copper soln. + 25 c.c. alk. tartrate soln. + 50 c.c. water in a 400 c.c. beaker to 100°C. in exactly 4 minutes.

[2] The finely divided, long-fibred asbestos to be used in the glucose determination should be digested with nitric acid, washed, then digested with sodium hydroxide and washed. When gooch filters are prepared, they should be washed with boiling Fehling's solution, then with nitric acid. The mats thus prepared can be used for a long time.

strong alcohol and 200 c.c. of distilled water for a day or two at ordinary temperature; the filtered solution is employed as indicator.

Determination.

Place 0.7 grm. of leather in a digestion flask. Add 10 grm. of powdered potassium sulphate and from 15 to 25 c.c. (ordinarily about 20 c.c.) of concentrated sulphuric acid. Place the flask in an inclined position and heat below the boiling point of the acid from 5 to 15 minutes or until frothing has ceased (a small piece of paraffin may be added to prevent extreme foaming).

Then raise the temperature and boil briskly until the liquid has become quite clear and nearly colourless (the digestion should take from 4 to 5 hours).

After cooling, dilute with about 200 c.c. of water. Next add 50 c.c. of soda solution, or sufficient to make the reaction strongly alkaline, pouring it down the side of the flask so that it does not mix at once with the acid solution. Connect the flask with the condenser, mix the contents by shaking, and distil until all ammonia has passed over into the standard acid. The first 150 c.c. will generally contain all the ammonia. The operation usually requires from 40 minutes to 1½ hours. The distillate is then titrated with standard alkali.

Previous to use, the reagents should be tested by a blank experiment with sugar, which will partially reduce any nitrates present that otherwise might escape notice.

Provisional Method of Colour Valuation of Tanning Materials.

Immerse a piece of thoroughly wetted white broadcloth, 3 by 4 in. in size, in a solution of the material to be tested containing 3% of tannin, and allow to remain with frequent agitation for 45 minutes. The solution previous to immersing the cloth is heated on a water-bath to 50° C. and the source of heat then removed, the colouring being effected without a continuance of heating. (Care must be taken that the temperature of the bath is not greater than that of the solution, *i.e.*, 50° C.) The solution, in volume 250 c.c. should be contained in a porcelain or glass beaker not less than 3½ in. in diameter and 4 in. deep, and the beaker immersed at least 2½ in. in the water. The bath should not be exposed to rapid cooling (5° being the usual drop) during the test. At the expiration of the time of immersion, the cloth is removed from the solution and the free colouring matter washed out thoroughly in water heated to 50° C., then well squeezed in the hand and further excess moisture removed by rolling for a minute or two in a clean towel. It is then dried smooth between pieces of blotting-paper in a letter press.

Newman's process of extracting the fat from fodders has been utilised by E. Golberg[1] for leather. Extraction takes place in the cold with tri-

[1] *Ledertech. Rundschau*, 1912, 49.

chlorethylene. Slightly higher results are obtained than by extraction with carbon disulphide in the Soxhlet apparatus; 10 grm. of leather are shaken with 100 c.c. of the solvent for 1 hour, and after filtering 50 c.c. are evaporated in an extraction flask to constant weight. The greatest difference between the two methods was (in one case) 0.05%.

The influence of the position of the sample taken from a hide for analysis has been emphasised by C. R. Oberfell.[1] Variations up to 4% in the amount of hide substance present may be obtained when samples are taken from the butt and shoulder respectively.

Under American conditions, weighting or loading leather is practised up to 16% when both glucose and Epsom salts are present. In France, leather may not be sold with an ash exceeding 1.5%. In an extended enquiry by Veitch and Rogers[2] it was observed that no less than 63% of the leathers examined (American) were loaded in this manner. The following particulars give in detail the results obtained: See pp. 415 to 417.

The presence of sulphite-cellulose and its detection in leather has been dealt with by W. Moeller.[3]

Analysis of East Indian Tanned Hides.—M. C. Lamb[4] has given many analyses indicating that on the whole these tanned hides are fairly pure. In a typical example the following results were obtained:

Leather fibre... 68.86 %
Oil and fatty matter.. 8.43 "
Water soluble matter.. 8.6 "
Moisture.. 13.7 "
Mineral matter... 1.12 "

Procter also estimates the insoluble residue after extraction and estimates the nitrogen in this to determine the actual hide substance present.

The nature of the tannin material in the leather is determined by Kohnstein[5] by boiling a portion of the water extract from the leather under a reflux condenser with formaldehyde and hydrochloric acid and filtering the solution after well cooling. The usual test with iron alum and sodium acetate will indicate the presence of pyrogallol tannin. Quebracho or mimosa tannin may be recognised by placing a drop of sulphuric acid on a rod moistened with extract when in their presence a purple colour developing to violet will be noticed.

Chrome Leather.—In the analysis of chrome leather which contains no tannin certain modifications are necessary. The leather may be pulverised and extracted with petroleum ether. After drying it is extracted with 90% alcohol for 3 to 4 hours. The leather is then extracted with water in the same way as ordinary leather. The petroleum ether extract contains the non-saponifiable fats and any free sulphur. Nitrogen is determined in the usual

[1] J. A. L. C. A., 1912, 7, 127.
[2] Bull. Dept. Agric. Bureau of Chemistry, 1914.
[3] Collegium, 1914, 531, 489.
[4] Tanners Year Book, 1913, 165.
[5] Allgem. Geber. Zeit., 1912, 14, 5.

ANALYSIS OF AMERICAN LEATHERS.

Place sample was obtained	Time sample was obtained	Description of sample	Tannage claimed	Moisture, per cent.	Ash, per cent.	Petroleum ether extract, per cent.	Water-soluble material			Epsom salts, per cent.	Glucose, per cent.	Combined tannins, per cent.	Hide substance, per cent.	Ratio of combined tannins to hide substance
							Tannins, per cent.	Non-tannins, per cent.	Total soluble, per cent.					
Boston, Mass.	1906	Sole, supposed to be weighted.	Oak	7.5	0.95	1.5	12.7	14.0	26.7	....	5.4	26.1	37.9	0.69
Do.	1907	No. 1, Texas oak sole	Do.	6.9	0.8	4.9	17.0	6.8	23.8	....	1.5	24.7	39.4	0.63
Do.	Do.	No. 2, Texas oak sole	Do.	6.5	2.1	1.7	10.2	18.9	29.1	3.9	5.7	30.9	31.5	0.98
Do.	Do.	No. 3, Hemlock sole	Hemlock	7.3	1.9	2.2	14.8	11.9	26.7	1.5	7.2	35.8	37.7	0.68
Do.	Do.	No. 4, Oak sole	Oak	6.9	1.2	4.0	15.1	6.4	21.5	0.5	2.6	29.2	38.1	0.77
Do.	Do.	No. 5, English pure bark	....	7.3	2.3	1.3	14.2	11.3	25.5	0.7	0.7	27.0	38.6	0.70
Do.	Do.	No. 6, Hemlock sole	Hemlock	7.7	1.0	1.0	17.7	11.5	29.2	1.2	4.6	24.1	37.4	0.64
Baltimore, Md.	Do.	Oak sole	Oak	6.8	2.6	2.5	14.9	18.4	33.3	4.7	12.0	21.4	37.2	0.58
Do.	Do.	Union sole	Union	8.3	2.7	1.5	13.2	14.8	28.0	6.4	6.1	27.5	33.6	0.82
Do.	Do.	Texas oak sole	Oak	6.3	1.7	1.5	13.7	13.1	26.8	4.0	5.3	28.5	34.6	0.80
Do.	Do.	Hemlock sole	Hemlock	7.3	1.8	0.4	7.5	19.2	26.7	4.6	10.0	29.5	36.8	0.80
Do.	Do.	Texas scoured oak	Oak	7.6	0.7	2.0	14.8	7.1	21.9	0.4	2.3	28.0	40.5	0.69
Boston, Mass.	Do.	Drum tanned	....	7.9	0.9	0.7	13.4	2.9	16.3	1.2	0.0	26.1	49.0	0.53
Do.	Do.	Do.	....	8.8	0.9	1.0	10.2	3.2	13.4	0.4	0.2	27.5	49.9	0.55
Unknown	Do.	No. 1 sole, No. 2 bend	Union	4.4	1.9	3.2	14.4	16.1	30.5	4.4	9.2	23.8	33.4	0.71
Alexandria, Va.	Do.	Scoured oak sole, No. 1 bend.	Oak	3.6	0.3	4.7	15.6	4.6	20.2	....	1.2	33.0	37.4	0.88
Do.	Do.	Do.	Do.	3.8	0.3	3.3	14.9	5.1	20.0	....	1.8	32.5	40.3	0.81
Do.	Do.	Scoured oak, extract tanned (in wheel after vat).	Do.	4.9	0.2	3.5	14.2	5.0	19.2	1.4	1.5	33.3	39.9	0.83
Do.	Do.	Vat-tanned belting, No. 1 butt.	Hemlock	5.8	0.9	5.3	11.5	5.5	17.0	2.4	1.3	34.5	38.0	0.91
Do.	Do.	No. 2 side	Union	5.9	1.2	3.3	14.8	12.1	26.9	3.0	6.2	28.0	35.7	0.78
Washington, D. C.	Do.	Oak	Oak	7.0	1.0	4.5	14.2	9.7	23.9	....	2.7	30.8	34.6	0.89
Do.	Do.	Scoured oak	Do.	6.3	0.6	5.5	13.6	10.8	24.4	2.1	2.5	27.7	36.6	0.77
Do.	Do.	Do.	Do.	5.5	1.0	3.7	13.2	6.8	20.0	6.1	1.5	32.4	35.8	0.89
Do.	Do.	Do.	Do.	8.2	1.9	4.4	13.5	7.7	21.2	4.4	2.0	28.0	39.8	0.70
Do.	Do.	Do.	Do.	6.7	1.4	2.9	12.6	17.9	30.5	1.0	7.2	28.8	35.0	0.77
Do.	Do.	Hard-rolled scoured oak	Do.	6.9	1.5	2.3	15.4	15.0	30.4	0.5	5.5	25.9	32.5	0.80
Do.	Do.	Do.	Do.	7.8	1.5	2.5	14.9	12.9	27.8	2.8	4.4	27.5	33.9	0.81
Do.	Do.	Scoured oak	Do.	8.9	2.4	3.1	10.1	12.5	22.6	4.1	6.1	27.7	40.0	0.69
Do.	Do.	Do.	Do.	8.0	1.4	1.2	9.3	10.7	20.0	0.4	5.1	26.4	42.5	0.62
Do.	Do.	Do.	Do.	7.7	0.5	1.5	12.5	14.9	27.4	0.6	4.0	30.0	32.2	0.93
Do.	Do.	Texas scoured oak	Do.	7.6	0.4	4.1	13.4	11.6	25.0	0.4	2.6	29.5	35.5	0.83
Do.	Do.	Do.	Do.	9.4	1.0	3.6	15.3	6.5	21.8	3.2	0.8	25.1	40.7	0.62
Do.	Do.	Scoured	Do.	8.9	0.9	4.4	15.3	2.4	17.7	2.8	2.8	29.1	41.6	0.70
Do.	Do.	Do.	Do.	8.1	0.5	3.6	9.4	10.9	20.3	2.3	2.1	26.7	40.7	0.66
Do.	Do.	Do.	Do.	8.0	0.4	1.9	11.6	8.5	20.1	1.0	2.1	23.3	43.8	0.53
Do.	Do.	Scoured oak sole	Do.	7.7	0.9	1.0	14.7	6.7	21.4	1.2	0.5	30.9	37.4	0.82
Do.	Do.	Do.	Do.	7.7	0.4	1.5	10.9	7.6	18.5	....	1.2	31.1	40.9	0.76

ANALYSIS OF AMERICAN LEATHERS.—(*Continued.*)

Place sample was obtained	Time sample was obtained	Description of sample	Tannage claimed	Moisture, per cent.	Ash, per cent.	Petroleum ether extract, per cent.	Water-soluble material			Epsom salts, per cent.	Glucose, per cent.	Combined tannins, per cent.	Hide substance, per cent.	Ratio of combined tannins to hide substance
							Tannins, per cent.	Non-tannins, per cent.	Total soluble, per cent.					
Baltimore, Md...	1912	Scoured oak...	Do.	7.2	1.4	1.6	14.8	19.5	34.3	4.9	9.6	23.6	33.0	0.72
Do.	Do.	Do.	Do.	7.4	1.0	4.1	13.1	14.8	27.9	2.1	6.0	25.3	35.0	0.72
Do.	Do.	Texas scoured oak...	Do.	8.2	0.6	3.4	11.9	7.5	19.4	0.3	1.6	32.3	36.4	0.88
Do.	Do.	Do.	Do.	8.9	0.4	2.0	13.6	5.9	19.5		1.4	30.0	39.3	0.76
Do.	Do.	Scoured oak...	Do.	8.8	1.5	1.0	13.6	18.7	32.3	4.5	3.5	25.2	32.4	0.78
Do.	Do.	Hemlock sole...	Hemlock.	8.3	2.5	1.3	9.3	22.1	31.4	5.7	12.4	25.7	33.0	0.78
Do.	Do.	Do.	Do.	7.6	1.6	1.6	9.7	18.1	27.8	6.4	6.4	24.2	38.5	0.63
Do.	Do.	Union sole...	Union.	8.7	2.0	2.5	14.6	13.4	28.0	4.1	5.5	27.4	33.1	0.83
Do.	Do.	Do.	Do.	7.4	1.2	3.7	13.5	11.0	24.5	3.8	5.4	28.5	35.6	0.80
Do.	Do.	Hemlock sole...	Hemlock.	8.4	0.9	1.1	12.0	17.5	29.5	2.8	10.5	28.9	31.7	0.91
Do.	Do.	Do.	Do.	7.7	0.3	1.1	14.1	14.9	29.0	2.0	8.2	27.3	34.6	0.78
Do.	Do.	Scoured oak...	Oak.	8.2	0.3	1.3	13.7	6.1	19.8		1.5	33.1	37.3	0.89
Do.	Do.	Do.	Do.	8.1	0.4	1.1	14.0	5.9	19.9		1.2	31.3	39.2	0.80
Do.	Do.	Do.	Do.	12.3	0.5	0.9	10.9	7.1	18.0		1.2	28.9	39.6	0.73
Do.	Do.	Do.	Do.	7.9	0.5	0.7	10.5	7.3	17.8		1.5	32.4	40.9	0.79
Do.	Do.	Do.	Do.	8.4	1.2	1.1	13.2	14.5	27.7	1.9	5.9	27.0	35.5	0.76
Do.	Do.	Vat (no extract) scoured oak.	Do.	8.6	0.9	2.3	12.0	12.6	24.6	2.5	5.6	27.0	37.2	0.73
Do.	Do.	Do.	Do.	7.5	0.3	2.9	11.5	6.1	17.6		1.8	29.5	42.2	0.69
Do.	Do.		Do.	7.6	0.3	2.6	12.9	4.7	17.6		0.5	29.0	42.9	0.68
Alexandria, Va...	Do.	Vat, chestnut oak extract, scoured oak.	Do.	7.9	0.3	1.9	12.6	4.6	17.2		0.4	34.2	38.5	0.89
Do.	Do.	Vat, chestnut oak extract, and quebracho...	Do.	7.3	0.4	2.4	14.4	4.7	19.1	0.3	0.5	34.4	36.5	0.94
Do.	Do.	Do.	Do.	7.9	0.4	3.7	12.4	5.5	17.9		0.4	33.3	36.9	0.90
Do.	Do.	Do.	Do.	8.2	0.2	1.5	14.4	6.3	20.7		0.6	34.4	34.9	0.99
Do.	Do.	Do.	Do.	8.4	0.7	1.8	13.8	5.8	19.6		0.5	34.8	35.1	0.99
St. Louis, Mo...	1909	No. 1, Texas oak...	Union.	5.8	0.7	3.6	17.3	9.8	27.1		2.7	25.4	37.8	0.67
Do.	Do.	No. 2, union...	Union.	5.1	2.4	5.6	14.7	18.9	33.6	7.5	6.5	25.1	30.3	0.83
Do.	Do.	No. 3, extract tanned, oak.	Oak.	4.1	1.9	5.6	12.9	13.1	26.0	3.5		27.1	36.9	0.73
Do.	Do.	No. 4, oak...	Do.	4.1	1.3	4.1	14.5	12.7	27.2	3.1	3.6	26.8	37.5	0.71
Do.	Do.	No. 5, extract tanned, union.	Union.	4.2	1.6	2.5	16.2	10.2	26.4	3.9	3.4	28.3	38.3	0.74
Do.	Do.	No. 6, extract tanned.	Do.	3.4	1.0	4.0	14.2	11.4	25.6	2.9	4.3	29.3	37.4	0.78
Do.	Do.	No. 7, weighted, hemlock.	Hemlock.	3.6	0.9	1.8	11.7	12.9	24.6	2.1		27.1	42.6	0.64
Do.	Do.	No. 8, hemlock...	Do.	4.7	0.7	1.9	11.6	17.1	28.7	2.1	5.9	29.7	34.7	0.86
Do.	Do.	No. 9, extract, union.	Union.	4.2	1.1	2.0	14.2	11.3	25.5	2.7	3.7	28.7	39.3	0.73
Do.	Do.	No. 10, adulterated, South American dry hide.												
Do.	Do.	No. 11, belly, oak...	Oak.	4.7	2.7	2.9	15.5	21.2	36.7	3.8	2.8	25.8	29.6	0.87
Do.	Do.	No. 12, extract, hemlock...	Hemlock.	5.6	0.5	3.8	11.4	13.0	24.4		3.7	25.7	40.4	0.64
Do.	Do.	No. 13, so-called oak...	Oak.	5.6	0.4	1.4	15.4	7.6	23.4			26.2	43.1	0.76
				6.3	1.5	3.5	9.2	12.2	24.4	4.7	3.6	27.3	38.2	0.71

ANALYSIS OF AMERICAN LEATHERS.—(Continued.)

Place sample was obtained	Time sample was obtained	Description of sample	Tannage claimed	Moisture, per cent.	Ash, per cent.	Petroleum ether extract, per cent.	Water-soluble material Tannin, per cent.	Non-tannin, per cent.	Total soluble, per cent.	Epsom salts, per cent.	Glucose, per cent.	Combined tannins, per cent.	Hide substance, per cent.	Ratio of combined tannins to hide substance
Do.	1909	No. 14, oak	Do.	4.3	0.7	3.4	6.1	10.6	16.7		5.2	29.1	46.2	0.63
Do.	Do.	No. 15, oak back	Do.	4.8	0.8	4.3	13.7	10.3	24.0		5.6	26.7	39.9	0.67
Do.	Do.	No. 16, weighted, hemlock	Hemlock	5.7	1.9	2.3	14.9	20.2	35.1	3.4	10.7	24.4	32.2	0.76
Do.	Do.	No. 17, hemlock	Do.	4.9	0.9	2.2	14.3	8.6	22.9	1.3	3.1	27.6	42.1	0.66
Do.	Do.	No. 18, South American dry hide, weighted		4.3	1.0	2.9	13.7	17.3	31.0	4.3	5.6	30.0	31.5	0.95
Do.	Do.	No. 19, best	Chrome[1]	4.0	6.7	32.1	0.2	2.5	2.7		1.4		47.0	
Do.	Do.	No. 20, best	Chrome[2]	2.9	6.0	34.6	0.2	2.9	3.1		1.4		54.9	
Portsmouth, Ohio.	Do.	No. 1	Union	5.9	1.5	3.1	13.4	14.5	27.9	4.9	5.5	26.4	36.4	0.73
Do.	Do.	No. 2, oak	Oak	5.7	0.6	2.1	10.4	13.5	23.9		8.6	25.6	42.4	0.60
Do.	Do.	No. 3	Union	5.2	0.8	2.5	13.3	9.7	23.2	2.6	2.8	30.1	38.0	0.79
Do.	Do.	No. 4	Oak	5.8	0.3	3.5	11.7	7.8	19.5		1.2	30.7	40.8	0.75
Do.	Do.	No. 5	Do.	5.6	0.4	1.4	12.0	7.6	19.6		1.5	31.0	41.9	0.74
Do.	Do.	No. 6	Do.	5.6	0.3	0.9	8.9	19.6	28.5	0.2	8.0	24.0	40.1	0.60
Do.	Do.	No. 7	Do.	6.4	0.3	1.3	12.2	7.0	19.2		0.9	29.4	43.4	0.68
Unknown.	Do.	No. 8, Sole, lot No. 110, iron, not treated	Union	4.8	0.9	2.5	12.1	9.8	21.9	0.2	3.8	28.0	42.5	0.66
Do.	Do.	Sole, lot No. 210, iron, treated about 6 per cent	Do.	5.9	0.4	4.7	9.8	3.7	13.5		1.0	28.1	47.5	0.59
Do.	Do.	No. 12	Do.	6.2	2.3	2.0	10.0	13.5	23.5	0.2	9.2	26.7	41.3	0.65
Do.	Do.	Dark color	Do.	5.6	0.8	1.2	12.0	12.1	24.6		0.8	27.3	41.0	0.67
Do.	Do.	Do.	Do.	5.3	0.2	4.3	12.2	5.8	18.0		1.4	36.4	35.7	1.01
Do.	Do.	Green yellow cast	Do.	5.4	0.3	2.9	10.7	5.9	16.6		1.0	40.3	34.5	1.16
Do.	Do.	Green yellow, redder than above	Oak	4.9	0.3	2.5	14.1	4.3	18.4		0.8	31.2	42.7	0.73
Washington, D. C.	Do.	Hemlock back	Hemlock	5.6	0.8	1.4	14.5	4.4	18.9		0.9	31.8	42.0	0.75
Alexandria, Va.	1910	Oak	Do.	5.6	1.2	3.2	14.4	13.9	28.3	1.2	9.7	32.4	30.2	1.07
Washington, D. C.	1911	Scoured oak sole	Oak	9.3	1.2	1.1	17.8	7.7	25.5		2.5	29.3	34.5	0.85
Do.	Do.	Flexible oak bend	Do.	7.2	0.4	1.9	13.9	5.3	19.2		0.7	34.0	37.9	0.90
Do.	Do.	Oak sole	Do.	8.2	0.7	3.9	11.7	18.5	30.2		10.2	23.5	33.9	0.69
Portsmouth, Ohio.	1912		Union	8.9	0.7	4.0	13.0	5.3	18.2	1.9	2.3	26.0	41.8	0.64
Do.	Do.		Do.	6.3	1.0	2.6	13.0	8.5	21.5	3.8	4.2	27.7	42.0	0.66
Do.	Do.		Do.	5.9	0.7	1.1	12.5	10.1	21.3	4.6	4.6	29.2	42.2	0.69
Do.	Do.		Hemlock	6.8	1.5	2.9	11.8	11.8	27.4	2.5	6.9	28.4	36.7	0.77
Do.	Do.		Oak	5.6	0.7	1.6	14.8	7.7	22.5	1.5	1.7	28.7	40.1	0.71
Average				6.6	1.1	3.4	12.8	10.5	23.3	2.7	3.8	28.5	38.2	0.75
Average in weighted samples										3.0	5.5			

[1] Sample No. 9713: 5.02 per cent. chromium trioxide in original sample.
[2] Sample No. 9714: 1.32 per cent. chromium trioxide in original sample; 0.30 per cent. chromium trioxide in water soluble.
[3] Procter allows anything over 2 % of glucose as adulteration.

manner.   To determine the chromium and aluminium, 5 grm. of the leather are fused with 4 grm. sodium carbonate for half an hour.   The chromium and aluminium are then present as chromate and aluminate and are estimated in the usual manner.   It has been pointed out by J. Thuau that the separation of chromium from aluminium should always be effected owing to the increased use of chromium as a fore-tannage in the case of alum-tanned leather.   Sometimes a light alum tannage also follows an all-chrome tannage.

Iron is determined in the usual manner if present.

ERRATUM IN VOL. V.

Page 105, line 6 for "Jeit" read "Zeit".

ANALYSIS OF COLOURING MATERIALS.

By W. P. DREAPER, F. I. C.

CHEMICAL EXAMINATION OF DYED FIBRES.

It is claimed by Holden[1] that direct and developed blacks on cotton may be distinguished from sulphide or aniline blacks by the following simple preliminary test:

The sample of dyed cloth is singed in a Bunsen flame. The part affected by the heat will change its colour. The substantive, developed or coupled class of blacks give a permanent light brown colouration at the singed portion while the sulphide and aniline black dyed cotton gives a faint black-brown colouration. It is claimed that the difference is easily distinguished and divides the blacks into two groups.

In the estimation of small quantities of dyestuffs more especially in foodstuffs W. E. Matthewson[2] oxidises with bromine water a few c.c. of the dye solution, the bromine being added gradually until the colour of the dye solution is destroyed. Hydrazine sulphate is then added to take up the excess of bromine and finally an excess of sodium carbonate. In a similar test a few drops of β-naphthol solution are added just before the sodium salt. Various colours result which have been recorded in the article referred to; in special cases the test might be of some value in textile work as a confirmation of other tests.

Physical Examination of Dyed Fibres.

Fastness of Colours.—Owing to the unsatisfactory character of the methods of determining the "fastness" of colours against light and other reagents the Society of German Chemists appointed in 1911 a committee to deal with this question. As a result of this decision the committee recommended in 1911, that for the classification of dyestuffs in order of fastness five standards or grades should be adopted, 1 representing the least and 5 the greatest fastness. For each grade in each kind of fastness a typical dyestuff was to be given[1].

Only the fastness of dyes on the fibre is dealt with, not that of the dyes themselves.

A sub-committee was appointed to prosecute further research, and the

[1] J. Soc. Dyers and Col., 1913, 29, 36
[2] Chem. News, 1913, 265.
[3] Chem. Zeit., 1914, 154.

results of the investigation were made known at a meeting of the section for dyeing and textile chemistry in May, 1912, when it was decided to increase the numbers of grades in the fastness to light section from five to eight.

In the selection of the typical dyestuff when a particular colour chosen was manufactured by several firms each product was submitted to the fastness tests.

A report of the committee was presented in Breslau in September, 1913, when it was resolved to publish the results of the investigation so far as it had then progressed.

1. **Fastness to Light.**—Parallel tests were made both open and under glass.

COTTON	WOOL
I.— 5.5 % Chicago Blue 6B, 1 bath.	I.—3.15 % Indigotine I.
II.— 1.0 % Methylene Blue BG.	II.—3.00 % Patent Blue A.
III.— 1.0 % Indoine Blue R.	III.—3.25 % Amaranth.
IV.—20.0 % Kryogen Violet 3R, 1 bath.	IV.—4.4 % Azo Acid Red B.
V.— 2.5 % Benzo Light Red 8BL. 1 bath.	V.—5.00 % Acid Violet 4RN.
VI.— 9.0 % Hydron Blue G paste, 1 bath.	VI.—2.5 % Diamine Fast Red F., chromed.
VII.— 8.0 % Kryogen Black, 1 bath.	VII.—4.00 % Anthraquinone Green GXN.
VIII.—25.0 % Indanthrene Blue GC paste, 1 bath.	VIII.——— Indigo Pure.

2a. **Fastness to Washing and Boiling of Dyed Cotton in Contact with White Cotton.**—(A) The sample is plaited with white cotton and treated for ½ hour at 40° C. in fifty times its amount of a 0.2% solution of Marseilles soap. The plait is then wrung out by hand, soaked in the soap solution and again wrung out, this being repeated ten times. The cotton is then washed and dried.

(B) This is a more severe test. The solution contains 0.5% of soap and 0.3% of sodium carbonate, and the cotton is kept in the boiling solution for ½ hour, then cooled to 40° C. and wrung as in A.

GRADE	TYPICAL DYESTUFF
I.—Treated as in A. Colour a little lighter, white cotton dyed.	I.—3 % Benzopurpurin 4B, dyed for 1 hour at the boil with 20 grm. of Glauber's salt cryst. to the litre. An equal quantity of Glauber's salt is then added and the dyeing continued for 15 minutes longer.
II.—Treated as in A. Colour unchanged, white cotton not at all or only slightly dyed.	II.—5 % Primuline dyed as in I., then diazotised and coupled with β-naphthol. Soaped with 0.2 % soap for 5 minutes at 35° C.
III.—Treated as in B. Colour a little lighter, white cotton only slightly tinted.	III.—2.5 % Indoine Blue BB on yarn previously mordanted with 6 % tannin and 3 % antimony salt. Dyed with addition of 5 % acetic acid ¼ hour cold, ¼ hour heating up to boiling, and ¼ hour at the boil.
IV.—Treated as in B. Colour unchanged, white cotton only faintly dyed.	IV.—12 % Immedial Indone R conc., dyed at the boil for 1 hour with 1½ times its weight of sodium sulphide cryst. in a bath containing 3 grm. soda and 20 grm. sodium chloride to the litre. After 1 hour an equal amount of sodium chloride is added and the dyeing continued for ¼ hour longer. Or Indigo dyed from a hydrosulphite bath.
V.—Treated as in B. Colour unchanged, white cotton not at all or only slightly tinged.	V.—Alizarin Red.

2b. **Fastness to Washing of Dyed Wool in Contact with White Wool or Cotton.**—(A) The sample, plaited with wool or cotton, is heated for ¼ hour at 40° C. with 50 times its amount of a solution containing 10 grm. of Marseilles soap and 0.5 grm. sodium carbonate in a litre, then wrung out five times with the hands, washed and dried.

(B) The same treatment at 80° C. for ¼ hour.

Fastness in Contact with Wool.

Grade	Typical Dyestuff
I.—Treated as in A. Colour much changed, pronounced bleeding to the white.	I.—2% Orange II dyed with 10% Glauber's salt cryst. and 10% sodium bisulphate for 1 hour at the boil.
III.—Treated as in A. Little or no change in colour; no bleeding to the white.	III.—2% Patent Blue A dyed with 10%. Glauber's salt cryst. and 3% acetic acid. Dyeing commenced at 40° C. and heated within 20 minutes to boiling, and kept at the boil for 1 hour. 2% H₂SO₄ added after 1/2 hour's boiling.
V.—Treated as in B. Little or no change in colour; no bleeding to the white.	V.—7% Palatine Chrome Black 6B dyed with 10% Glauber's salt cryst. and 3% acetic acid. Commenced at 60° C., raised to boiling in 15 minutes. After 1/2 hour's boiling 2% H₂SO₄ added, cooled to 70° C., 2.5% of K₂Cr₂O₇ added, and kept just at the boil for 40 minutes.

Fastness in Contact with Cotton.

I.—Treated as in A. Pronounced bleeding into the white.	I.—2% Chrysophenin G dyed with 10% Glauber's salt cryst. Entered at 40° C., raised to boiling in 20 minutes and kept at the boil for 1 hour. 2% acetic acid added after 3/4 hour boiling.
III.—Treated as in A. No bleeding into the white.	III.—2% Patent Blue A dyed as before.
V.—Treated as in B. No bleeding into the white.	V.—7% Palatine Chrome Black 6B dyed as before.

(3a) **Fastness to Water of Dyed Cotton.**—The sample is plaited with boiled-off white cotton, washed Zephyr wool, and white silk, taking two parts of the dyed sample to one part of the white material. It is allowed to remain for 1 hour in cold distilled water (about 20° C.) and then dried at the ordinary temperature.

Grade	Typical Dyestuff
I.—With a single treatment the colour is somewhat lighter and the white material coloured.	I.—2% Chrysophenin G dyed as in 2a, I.
III.—With a single treatment colour unaffected and white material not changed.	III.—2% Chloramine Yellow dyed like I.
V.—With three successive treatments (each with fresh water) colour unaffected and white material not changed.	V.—8% Immedial Carbon B dyed as in 2a. IV.

(3b) **Fastness to Water of Dyed Wool.**—Treatment as in 3a, but 12 hours' duration.

Grade	Typical Dyestuff
I.—With a single treatment colour changed and white material coloured.	I.—2% Azo Yellow dyed at the boil for 1 hour with 10% Glauber's salt and 10% sodium bisulphate.
III.—With a single treatment colour unchanged, no bleeding into the white.	III.—2% Patent Blue dyed as in 2b.
V.—With three successive treatments (each time with fresh water) colour unchanged, bleeding, none or very slight.	V.—7% Palatine Chrome Black 6B dyed as in 2b.

(4) **Fastness to Rubbing** (for all dyeings).—White unfinished cotton, cloth is rubbed vigorously with the sample ten times backwards and forwards over a length of 10 cm.

(5a) **Fastness to Ironing of Dyed Cotton.**—The sample is covered with a doubled sheet of thin white unfinished cotton cloth which is moistened with water (100% water), and is ironed with an iron of such a temperature that with the same pressure it will just begin to scorch a piece of white flannel. The ironing is continued until the moist covering is quite dry.

Grade	Typical Dyestuff
I.—Colour much altered, and bleeds into the white cloth.	I.—1.5% Methylene Blue B dyed for 1½ hours with 20 times the cotton weight of distilled water and 2% acetic acid. The cotton is first mordanted with 6% of tannin and 3% antimony salt.
III.—Colour a little changed, but does not bleed.	III.—1% Benzopurpurin 4B dyed as in 2a.
V.—Colour not changed and does not bleed.	V.—1% Chloramine Yellow C dyed as in 2a, I.

(5*b*) **Fastness to Ironing of Dyed Wool.**—The sample is pressed for 10 seconds with a hot iron of such a temperature that with the same pressure it does not scorch a piece of white flannel.

GRADE	TYPICAL DYESTUFF
I.—Colour much changèd, and does not return to the original shade on cooling.	I.—Magenta S dyed with 10% Glauber's salt cryst. and 10% sodium bisulphate for 1 hour at the boil.
III.—Colour apparently changed, but quickly returns to the original shade on cooling.	III.—2% Amaranth dyed with 10% Glauber's salt cryst. and 10% sodium bisulphate at 60° C., heated to the boil in 20 minutes and kept for 1 hour at the boil.
V.—No change in the colour.	V.—2% Tartrazine dyed with 20% Glauber's salt cryst. and 10% sodium bisulphate at the boil.

(6) **Fastness to Sulphur of Dyed Cotton and Wool.**—The sample, plaited with an equal quantity of wool is soaped for 5 minutes at the ordinary temperature in a ½% solution of Marseilles soap. It is then wrung out and introduced into an atmosphere charged with sulphur dioxide, where it remains for 12 hours.

Sulphur Fastness of Dyed Cotton.

GRADE	TYPICAL DYESTUFF
I.—Colour changed, white wool coloured.	I.—1% Diamond Magenta dyed like Methylene Blue (5a) on a tannin-antimony mordant.
III.—Colour slightly changed, white wool not coloured.	III.—1% Columbia Black FF extra dyed as in 2a, I.
V.—Colour and white wool unchanged.	V.—1% Diamine Black B dyed as in 2b, I.

Sulphur Fastness of Dyed Wool.

GRADE	TYPICAL DYESTUFF
I.—Fairly pronounced change in the colour, but scarcely any bleeding.	I.—2% Diamine Scarlet B dyed as in 2b, I.
III.—Slight change in the colour, scarcely any bleeding.	III.—2% Milling Red G dyed with 10% Glauber's salt cryst., 2% acetic acid, commencing at 30° C., heating to boiling in 20 to 30 minutes, and dyeing for 1 hour at the boil. After 3/4 hour boiling 3% acetic acid is added.
V.—No change in the colour, and little or no bleeding.	V.—2% Palatine Black A dyed with 10% Glauber's salt cryst., 10% sodium bisulphate. Beginning at 60° C., heating to boiling in 20 minutes, and dyeing for 1 hour at the boil.

(7*a*) **Fastness of Dyed Cotton to Perspiration.**—The sample is plaited with the same quantity of white boiled-off cotton, and is heated for 10 minutes at 80° C. in a solution of ammonium acetate containing 5 c.c. of neutral ammonium acetate (30%) in a litre of distilled water. It is then dried without washing.

GRADE.	TYPICAL DYESTUFF
I.—Colour lighter, white cotton strongly coloured.	I.—1% Chrysophenin G dyed as in 2a, I.
III.—Colour unchanged, white cotton coloured.	III.—1% Diamine Black BH directly dyed as above.
V.—Colour and white cotton remain unchanged.	V.—20% Indanthrene Blue RS paste.

(7*b*) **Fastness of Dyed Wool to Perspiration.**—(*A*) Treatment with sodium chloride solution. The sample is spotted with a 10% NaCl solution, dried at the ordinary temperature, and then well brushed.

GRADE	TYPICAL DYESTUFF.
I.—Marked change in the colour.	I.—2% Amaranth dyed with 10% Glauber's salt cryst., 10% sodium bisulphate, beginning at 60° C., raising in 20 minutes to boiling, and keeping at the boil for 1 hour.
III.—The colour is fairly strongly changed.	III.—2% Wool Green S dyed with 10% Glauber's salt and 10% sodium bisulphate for 1 hour at the boil.
V.—No change in the colour.	V.—2% Brilliant Crocein 3B dyed with 10% Glauber's salt and 10% sodium bisulphate, beginning at 60° C., raising in 20 minutes to the boil, and dyeing at the boil for 1 hour.

(*B*) Treatment with ammonium acetate. As with the cotton, but the sample is plaited with both wool and cotton.

GRADE.	TYPICAL DYESTUFF
I.—Colour not or only slightly changed, white wool and cotton are tinted.	I.—2% Azo Yellow dyed as in 3b.
III.—Colour not changed, white cotton unchanged, wool slightly dyed.	III.—2% Amaranth dyed as in 5b.
V.—Colour not changed, neither wool nor cotton dyed.	V.—7% Palatine Black 6B dyed as in 2b.

(8) **Fastness to Alkali of Dyed Cotton and Wool (Dust Fastness).**—10 grm. of quicklime and 10 grm. of 24% ammonia are made to a cream with 1 litre of water. The dyed sample is spotted with this, dried without washing at the ordinary temperature, and then well brushed.

Wool.

GRADE	TYPICAL DYESTUFF
I.—Much altered.	I.—2% Water Blue dyed with 10% sodium bisulphate, beginning at 60° C., heating to boiling in 20 minutes, and then boiling for about 3/4 hour.
III.—Fairly pronounced change in the colour.	III.—2% Amaranth dyed as in 5b.
V.—No change in the colour.	V.—7% Palatine Chrome Black 6B dyed as in 2b.

Cotton.

GRADE	TYPICAL DYESTUFF
I.—Much altered.	I.—1.5% Malachite Green conc. dyed with a tannin-antimony mordant as in 5a, I.
III.—Fairly pronounced change.	III.—1% Direct Deep Black E extra dyed as in 2a, I.
V.—No change.	V.—8% Diamine Black BH dyed as in 2a II.

(9*a*) **Fastness of Dyed Cotton to the Acid Boil.**—The sample is plaited with wool and cotton and boiled for 1 hour with forty times the amount of a solution of cream of tartar containing 10% of the latter on the weight of the cotton.

GRADE	TYPICAL DYESTUFF
I.—Colour only a little lighter, white wool dyed.	I.—2% Chloramine Yellow C dyed as in 2a I.
III.—Colour not or only slightly changed, white wool only slightly dyed.	III.—3% Primuline developed with β-naphthol as in 2a, IV.
V.—Colour unchanged, wool and cotton not dyed.	V.—8% Immedial Carbon B dyed as in 2a, IV.

(9*b*) **Fastness of Dyed Wool to the Acid Boil.**—The sample is plaited with washed Zephyr wool and treated in seventy times the quantity of a 0.25% solution of bisulphate of soda for 1½ hours at 90–92° C.

GRADE.	TYPICAL DYESTUFF
I.—Colour slightly changed, white wool dyed.	I.—2% Chrome Yellow D dyed with 10% Glauber's salt cryst. and 3% acetic acid, beginning at 60° C., and heating to boiling in 15 minutes. The bath is kept at the boil for 1/2 hour, then 2% sulphuric acid is added, and, after cooling to 70° C., 1.25% of K₂Cr₂O₇. Finally, it is boiled gently for 30 minutes.
III.—Colour unchanged, white wool only slightly dyed.	III.—2% Diamine Scarlet B dyed as in 2b, I.
V.—Colour unchanged, white wool not at all or only very slightly tinted.	V.—6% Alizarin Black WX extra paste dyed with 10% Glauber's salt and 5% acetic acid. First at 60° C., heated to boiling in 20 minutes; after 1/2 hour boiling 5% acetic acid added. After a further 20 minutes' boiling the bath is cooled to 70° C., 2% K₂Cr₂O₇ added, and the bath gently boiled for 40 minutes.

(10) **Fastness to Acid of Dyed Cotton.**—The sample is spotted with mineral acids (10% sulphuric acid) and with organic acids (30% acetic),

and the change in shade compared with that of a place spotted with pure water.

GRADE	TYPICAL DYESTUFF
I.—With mineral acids strongly, with organic acids only slightly changed.	I.—3 % Diamine Scarlet B.
III.—With mineral acids strongly, with organic acids not changed.	III.—0.5 % Chrysophenin G.
V.—With mineral acids or organic acids no change.	V.—20 % Indanthren Blue RS paste.

(11) **Fastness of Dyed Cotton to Scouring.**—The dyed cotton is treated with the same weight of unbleached undyed cotton. Two methods of testing are adopted: (A) Scouring in an open boiler with 7.5% Na_2CO_3, 0.5% Marseilles soap, and 1% Ludigol calculated on the weight of the material; proportion of material to liquor 1 : 5; duration of operation, 6 hours. (B) The same process without the Ludigol.

GRADE	TYPICAL DYESTUFF
I.—By treatment A completely destroyed.	I.—Congo Red.
II.—By treatment A almost completely destroyed.	II.—Methylene Blue B.
III.—By treatment A very much changed.	III.—Indigo.
IV.—Resists treatment A.	IV.—Helindone Orange R.
V.—Resists treatment B.	V.—Turkey Red.

(12) **Fastness of Dyed Cotton to Chlorine.**—The sample is plaited with the same quantity of boiled-off white cotton and treated for 1 hour at about 15° C. in a freshly prepared bath of chloride of lime containing 1 grm. of active chlorine to the litre, or of sodium hypochlorite containing the same percentage of active chlorine and not more than 0.3 grm. of soda to the litre.

GRADE	TYPICAL DYESTUFF
I.—With sodium hypochlorite, colour lighter, bleeding to the white. With calcium hypochlorite, much lighter, bleeding into white.	I.—1 % Methylene Blue B dyed with a tannin-antimony mordant as in 5a.
II.—With sodium hypochlorite, colour changed but does not bleed. With calcium hypochlorite, much changed but does not bleed.	II.—6 % Indanthrene Olive G powder, dyed with 20 times the cotton weight of distilled water with addition of 5 times the dyestuff weight of NaOH (76 Tw.) and 2½ times of hydrosulphite for ½ hour at 60° C.
III.—With sodium hypochlorite, colour a little lighter, no bleeding. With calcium hypochlorite, much lighter, no bleeding.	III.—Indigo as in 2a, I.
IV.—With sodium hypochlorite, colour not changed, no bleeding. With calcium hypochlorite, somewhat lighter, no bleeding.	IV.—10 % Hydron Blue G paste dyed with half the weight of NaOH (76 Tw.) and half the weight of hydrosulphite. Otherwise as Indan-threne Olive (II.).
V.—Colour unchanged with both reagents, no bleeding.	V.—Ordinary Turkey Red dyeing.

(13) **Fastness to Mercerising of Dyed Cotton.**—Yarn dyed with the colouring matter to be tested is sewn into bleached unfinished cotton cloth and treated for 5 minutes in cold sodium hydroxide of 52° Tw.

I.—Colour slightly changed, some bleeding into the white.	I.—4 % Primuline developed with β-naphthol as in 2a.
III.—Colour unchanged, very slight tinging of white.	III.—1 % Chloramine Yellow C dyed as in 2a, I.
V.—Colour unchanged, no bleeding.	V.—8 % Immedial Carbon B dyed as in 2a, IV.

(14) **Fastness to Bleaching of Dyed Wool.**—White threads of wool, cotton, and silk are sewn into the dyed sample of woollen cloth, which is then treated with hydrogen peroxide. The bleaching bath is prepared with

100 parts of distilled water and 20 parts of hydrogen peroxide (10–12% vol.), and this solution is made just alkaline with ammonia. The bath must remain weakly alkaline (to Congo Red paper) throughout the operation. The sample is brought into the bath at an initial temperature of 45–50° C., and is allowed to remain for 12 hours in the gradually cooling liquid.

GRADE	TYPICAL DYESTUFF
I.—Colour only slightly changed, but bleeds slightly into the wool, silk, and cotton.	I. —2% Azo Yellow dyed as 3b.
II.—Colour is lighter and bleeds slightly into the wool, silk, and cotton.	II.—2% Patent Blue A dyed as in 2b.
III.—Colour is lighter but does not bleed.	III.—2% Fast Yellow S dyed as for Azo Yellow in 3b.
IV.—Colour not or only slightly changed, bleeds a little into the silk and cotton but not to the wool.	IV.—2% Chrysophenin G dyed as in 2b.
V.—Colour not or only slightly changed. Does not bleed, or only very slightly.	V.—2% Sulphocyanin GR extra dyed with 2% Glauber's salt cryst. and 5% ammonium acetate, commencing at 40° C., heating within ½ hour to 80–90° C., and keeping at this temperature for ¾ hour.

(15) **Fastness to Milling of Dyed Wool.**—(A) Neutral milling The sample is plaited with an equal quantity of white wool and cotton and then treated at 30° C. in forty times the quantity of a milling liquor containing 20 grm. of Marseilles soap to the litre. The sample is well worked with the hand, this being continued at intervals for 2 hours.

(B) The sample is treated at 40° C. in a solution containing 20 grm. Marseilles soap and 5 grm. sodium carbonate per litre.

With White Wool.

GRADE	TYPICAL DYESTUFF
I.—Treated as in A. Pronounced change in the colour and bleeding.	I.—2% Azo Yellow dyed as in 3b.
II.—Treated as in A. Slight change in the colour, slight bleeding.	II.—2% Ponceau RR dyed as for Orange II. in 2b.
III.—Treated as in A. No change or only slight in the colour, no bleeding.	III.—6% Sulphocyanin Black 2B dyed as in 14, V.
IV.—Treated as in B. No change or only slight in the colour, slight bleeding.	IV.—2% Chrome Yellow D dyed as in 9b.
V.—Treated as in B. Colour not or only slightly changed, no bleeding.	V.—7% Anthracene Chrome Black P extra dyed as in 2b, V.

With White Cotton.

GRADE	TYPICAL DYESTUFF
I.—Much bleeding into the white.	I.—2% Diamine Scarlet B dyed as in 2b.
II.—Slight bleeding into the white.	II.—2% Ponceau RR dyed as for Orange II. in 2b.
III.—No bleeding.	III.—6% Sulphocyanin Black 2B dyed as in 14 V.
IV.—Slight bleeding.	IV.—5% Diamond Black F chromed with 1.5% K₂Cr₂O₇ after dyeing as in 2b.
V.—No bleeding.	V.—7% Diamond Black PV dyed as in 2b.

(16) **Fastness to Carbonisation (Wool).**—The sample is soaked in sulphuric acid of 7.4° Tw., pressed so as to contain 100% of the solution, then dried at 80° C. for ¼ hour. The sample is then washed with 200 times its amount of distilled water for ¼ hour, and then for ¼ hour in a 0.2% solution of sodium carbonate. Finally, it is washed with water until neutral.

GRADE	TYPICAL DYESTUFF
I.—Strong change in the colour.	I.—2 % Alizarin Red W powder dyed on wool previously mordanted by boiling for 1½ hours with 3 % K$_2$Cr$_2$O. and 2½ % sodium bisulphate. The dyeing is done in a fresh bath with 2 % acetic acid added. Commenced at 30° C., heated to boiling in ½ hour, and kept at the boil for 1½ hours.
III.—Slight change in the colour.	III.—2 % Orange IV dyed with 10 % Glauber's salt cryst., and 10 % sodium bisulphate for 1 hour at the boil.
V.—Colour unchanged or only slightly changed.	V.—2 % Palatine Scarlet A dyed with 10 % Glauber's salt cryst., 10 % sodium bisulphate, at a commencing temperature of 60° C., heating in 20 minutes to, and keeping for 1 hour at the boil.

(17) **Fastness of Dyed Wool to Potting.**—*A.* The sample is plaited with the same quantity of white wool and cotton and treated for 2 hours in sixty times the amount of hot distilled water at 90° C.

(*B*) Like A with distilled water containing 1 grm. of Marseilles soap to the litre.

GRADE	TYPICAL DYESTUFF
I.—Treated as in A. The colour is changed, white wool or cotton strongly coloured.	I.—2 % Patent Blue A dyed as in 2b.
III.—Treated as in A. The colour is not or is only slightly changed, slight colouring of the white wool or cotton.	III.—5 % Diamond Black F dyed as in 2b, V., but chromed afterwards with 1½ % K$_2$Cr$_2$O$_7$.
V.—Treated as in B. Colour not changed at all or only slightly, no bleeding.	V.—7 % Alizarin Black WX extra dyed as in 2b. V.

(18) **Fastness of Dyed Wool to Steam Pressing.**—*A.* The sample is rolled on a steam cylinder and steamed for 5 minutes in the closed apparatus at 14 pounds pressure.

(*B*) The same for 10 minutes at 35 pounds pressure.

GRADE	TYPICAL DYESTUFF
I.—Treated as in A. Fairly strong change in the colour.	I.—2 per cent. Sulphocyanin GR extra dyed as in 14. V.
III.—Treated as in A. No change in the colour.	III.—2 per cent. Crocein AZ dyed like Brilliant Crocein 3B in 7b.
V.—Treated as in B. No change in the colour.	V.—9 per cent. Naphthol Black 6B dyed with 10 % sodium bisulphate. Dyeing commenced at 40° C., brought to boiling in 30 minutes, and dyed at the boil for 1 hour.

(19) **Fastness of Dyed Wool to Seawater.**—The sample is plaited with the same quantity of white wool and allowed to stand for 24 hours in forty times the quantity of a cold solution of 30 grm. NaCl and 6 grm. CaCl$_2$ in the litre, then dried without washing.

GRADE	TYPICAL DYESTUFF
I.—Colour only slightly changed, much bleeding to the white.	I.—2 % Chrysoin dyed as in 3b, I.
III.—Colour not at all or only slightly changed, some bleeding to the white.	III.—2 % Cyanol extra dyed as in 2b, III.
V.—Colour unchanged, no bleeding.	V.—6 % Sulphocyanin Black dyed as in 14. V.

The above tests must be used with great care by the general analyst. They are purely empirical, as the dyestuffs used as standards are only of commercial purity.

COLOURING MATTERS OF NATURAL ORIGIN.

By W. M. GARDNER, M.Sc., F. I. C.

The natural dyestuffs are used in slowly diminishing quantity and a few only are now of commercial importance. With the exception of indigo, no natural dyestuff has received much attention from the analytical point of view in recent years, but considerable work has recently been published on the estimation of this product

Indigo.

An exhaustive paper dealing with the estimation of indigo on dyed wool materials has been published by Green, Gardner, Lloyd and Frank.[1] It comprises: I. a critical examination of all previously published methods; II. a description of new methods for the quantitative estimation of indigo by weight; and, III. methods for the determination of the proportion of the total depth of shade which is due to indigo when other dyestuffs have been used in conjunction.

I. *Examination of Known Methods of Analysis.*—With the object of submitting these methods to critical investigation it was decided in the first place to employ, instead of indigo-dyed materials, pieces of undyed woollen cloth, or woollen cloth dyed with colouring matters usually employed for topping or bottoming, in which were wrapped weighed quantities of pure indigotin. Such cloth was then submitted to the extraction methods recommended by the various authors, and the recovered indigo either weighed as such or submitted to sulphonation with concentrated sulphuric acid at 70° C. and estimated by titration with $N/50$ potassium permanganate.

Experiments with Rawson's Hydrosulphite Method.—The indigo is separated from the cloth by reduction with an alkaline solution of sodium hydrosulphite, precipitated from the solution by aeration, filtered off, and weighed or estimated volumetrically, after sulphonation, by titration with permanganate.

The indigo separated by this method is apparently very pure, but the process is tedious, a large volume of liquid having to be filtered. The method gives moderately good results with lightly dyed materials, but somewhat variable results with heavily dyed, thick, felted cloths.

[1] *J. Soc. Dyers and Colourists,* 1913, 29, 227–241.

The method has been compared by Binz and Rung[1] with the acetic acid extraction method (which follows), and they find that the latter is less troublesome and more rapid, whilst the results are somewhat higher.

Experiments with Brylinski's Method.—Brylinski[2] extracts the material in a Soxhlet apparatus with glacial acetic acid, afterwards diluting the solution with water, filtering off the precipitated indigo on a weighed filter, washing with alcohol and ether, and finally drying and weighing. The method was improved by Binz and Rung[3] who dilute the acetic acid extract with a smaller quantity of water and mix in a separating funnel with ether. The indigo becomes suspended in the ether, leaving the aqueous acetic acid layer almost clear. The indigo can then be readily separated from the ether by filtration through a hardened filter paper, washed with alcohol and ether, dried, and weighed. Binz and Rung have found that a portion of the indigo is decomposed during the long boiling necessary for the extraction, and this they attribute to the reducing action of the wool. The authors have however proved that the loss is due to the decomposition by heat of indigo which has crystallised out upon the sides of the boiling flask.

The use of paper, even parchmentised paper, for filtration was found objectionable, (1) on account of its liability to vary in weight, and (2) because the precipitated indigo cannot be subjected to sufficiently rigorous treatment with reagents to remove impurities.

Experiments with Möhlau and Zimmerman's Method.—Möhlau and Zimmerman[4] are credited with having simplified and shortened the method of estimating indigo on the fibre by means of acetic-sulphuric acid. In this method, 10 grm. of the material, which is cut as fine as possible, are heated in a flask or beaker, on a rapidly boiling water-bath, with 100 c.c. of acetic-sulphuric acid (100 c.c. glacial acetic acid and 4 c.c. concentrated sulphuric acid) for about half an hour, shaking occasionally. The hot solution is filtered through a Gooch crucible, using hardened filter paper, the residue being repeatedly heated on the water-bath with acetic-sulphuric acid and filtered until the filtrate is no longer blue. The extract is warmed to 50° to redissolve the indigo, and is then diluted to twice its volume with boiling water. After cooling, the indigo is filtered off on a weighed, hardened filter paper, well washed with hot water until the filtrate is no longer acid, then with a little alcohol, and finally with 100 c.c. of ether, dried at 110°, and weighed. From the percentage of indigo obtained there is deducted for cotton materials 0.22%, this being the amount of modified cellulose supposed to be present with the indigo. With woollens no correction is considered necessary, since it is assumed that the dissolved wool remains in solution on dilution.

[1] *Zeit. angew. Chem.*, 1898, 904.
[2] *Rev. Gen. Mat. Col.*, 1898, 54; 1899, 5; *J. Soc. Dyers and Colourists*, 1898, 75.
[3] *Zeit. angew. Chem.*, 1898, 904.
[4] *Zeit. Farb. Text. Chem.*, 1903, 189.

This method gives very variable results, and the difficulty of manipulation is greatly increased when certain topping or bottoming colours are present, *e.g.*, logwood, myrabolans, etc.

Experiments with Other Proposed Extraction Methods.—The extraction of indigo from the fibre by solvents has generally been carried out in a Soxhlet apparatus, and the following substances, in addition to acetic acid, have been proposed for the purpose: Phenol, aniline,[1] naphthalene,[2] and nitrobenzene.[3] In using these solvents for extracting weighed quantities of pure indigo from wool, low results are obtained. The largest percentage error was obtained with nitrobenzene; naphthalene came next, then aniline, and lastly phenol. From this it appears that the chemical properties of the respective solvents, as well as their boiling points, play an important rôle in the quantitative extraction of the indigo. None of the above solvents is as suitable as acetic acid.

The extraction of indigo from dyed materials by solvents, followed by sulphonation and estimation of the indigo by examining the colour of the solution tintometrically (in place of titration with permanganate) was also found unsatisfactory, concordant tintometric or colourimetric readings being very difficult to obtain.

II. New Methods of Analysis.—In the hope of finding a solvent capable of effecting a more rapid extraction of the indigo from the dyed material, whilst at the same time leaving unaffected any topping or bottoming colour which might be present, experiments have been made by the authors with numerous organic liquids. The following are the results obtained:

(*a*) Pyridine (pure or commercial) is a valuable solvent for the extraction of indigo. It removes the indigo quantitatively and more rapidly than does acetic acid, leaving the wool in better condition and with less loss of wool substance. In a number of cases in which the topping or bottoming colours are removed by acetic acid, they are but little affected by pyridine.

(*b*) Piperidine extracts indigo quantitatively, and has practically the same properties as pyridine, but is much more expensive.

(*c*) Anisole also extracts indigo quantitatively, but lacks sufficient solvent power for practical use.

(*d*) Epichlorhydrin dissolves indigo slowly, giving a clear blue solution. The extraction though slow is quantitative.

(*e*) Dichlorhydrin dissolves indigo, giving a green solution. The indigo may be completely removed from dyed fabrics, but cannot be estimated by means of this solvent, as some decomposition takes place.

(*f*) Formic acid, 90%, extracts indigo more readily than does glacial acetic acid, but attacks the wool to a greater extent.

(*g*) Indigo is removed from the fibre, though not very readily, by

[1] Koenig, *Zeit. f. ang. Chem.*, 1889, 10.
[2] Schneider, *Zeit. Anal. Chem.*, 1895; *J. Soc. Dyers and Colourists*, 1895, 194.
[3] Gerland, *J. Soc. Chem. Ind.*, 1896, 17; 1897, 100.

chloroacetic ether; more slowly by amyl alcohol, amyl acetate, cumene, and perchlorethylene.

(h) Benzaldehyde removes indigo from the fibre very rapidly; at the same time combining with the indigo to form a soluble yellow compound. Many topping or bottoming colours, which are stripped from the fibre both by acetic acid and by pyridine, are but little affected if the indigo is removed by benzaldehyde. It is therefore useless for the estimation of indigo, but is of value for the rapid qualitative testing of a dyed cloth.

(i) Cresol has long been known as a good solvent for indigo, but it attacks the wool too seriously to be used at its boiling point. It has, however, proved a most satisfactory agent when diluted with about 25% of a neutral hydrocarbon, such as "solvent naphtha" or "turpentine substitute," so as to give a liquid which will extract in an ordinary Soxhlet extractor at a temperature of from 100° to 110°C. By this means the indigo can be removed completely in nearly all cases without disturbing the concomitant dyestuffs, which can then be examined or quantitatively determined.

Of the solvents experimented with, only glacial acetic acid, pyridine, benzaldehyde, and the cresol mixture appear to be capable of practical employment. The special use of each of these will be referred to later.

Improvements in Extraction Apparatus.—In the extraction method, as hitherto employed, the operation is unduly prolonged and frequently incomplete by reason of the fact that in the ordinary Soxhlet extractor the solvent is much below its boiling point when it comes into contact with the fibre.

It has been found that a great advantage is gained in extraction by solvents if means are taken to effect the extraction at the actual boiling point of the solvent instead of at the lower temperature usually obtained in the Soxhlet apparatus. By using the solvent at its boiling point, the time for extraction is greatly decreased. Thus, when using glacial acetic acid or pyridine as solvents, the thickest materials can be completely extracted in 4 hours at most, whilst ordinary materials do not require more than $1\frac{1}{2}$ to 2 hours. Extraction at the boiling point is effected by employing one of the forms of extractor which have been devised by L. L. Lloyd for this purpose (see Fig. 1, forms A, B, and C, on page 431).

If the form A is used the weighed cloth is placed loosely in the siphon tube, which is surrounded by an outer tube F. through which the vapour passes. If the cloth is packed too tightly, indigo separates in a crystalline form on the inner surface of the tube, and is only slowly redissolved. The solvent is condensed by an air or water condenser E., and flows into the siphon tube, from which it is intermittently siphoned over into the distilling flask D. The extraction is continued until a blue extract is no longer obtained. To detect whether the whole of the indigo has been extracted, the Bunsen flame is regulated so that the liquid in the extraction tube just fails to siphon over, and the solvent is kept in contact with the material for about 10 minutes.

If the extraction is complete, the liquid will not be coloured.   In the case of material which easily separates short fibres, it is preferable to pack it loosely in an inner tube drawn out at the end and provided with glass points to support it in the siphon tube.   Some crushed quartz is placed in the latter to act as filter, and to prevent short fibres from closing up the fine opening in the tube.

In the form of apparatus B, the cloth is placed loosely in the extraction
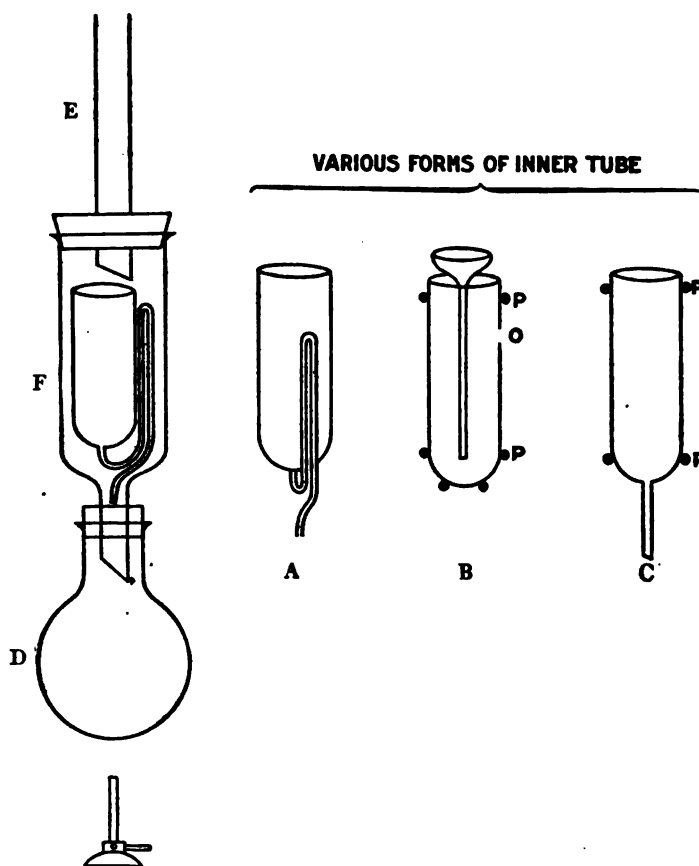
FIG. 15.

tube and the condensed solvent directed to the bottom by means of a long, drawn-out funnel tube, the solvent then overflowing from the side opening (o) in the extraction tube.

The simpler form of apparatus C is found to give good results for most cloths, and is easy to make.   The extraction tube is drawn out at the end, some glass points (P) being fused on to the outer surface so as to furnish a

passage round it for the vapour of the solvent. A little white wool, cotton wool, or crushed quartz is placed in the tube, as a filtering and regulating medium, and also to retain short fibres. The orifice and packing should be so adjusted as to prevent the condensed solvent from running through too quickly thus insuring the accumulation of a head of liquid above the cloth. This may be partially regulated by the flame and also by the packing of the cloth and filtering medium.

Lloyd finds the form of apparatus A the most satisfactory for most kinds of fabrics, but recommends the tube B for loosely woven and open cloths.

The form C is found by Frank to be suitable for most materials and is employed by Lloyd for loose wool, yarn, and other materials easily separated into fibres, the threads, if necessary, being held in position by covering with a small filter plate.

In the extraction of inidgo from heavily-dyed, thick, felted cloth, it is recommended to cut the material into small pieces or strips and to commence the extraction in the apparatus C, afterwards placing the tube containing the partially extracted material in the siphon tube A.

The weight of the cloth to be taken for gravimetric estimations varies from 3 to 15 grm., viz., sufficient to give from 0.03 to 0.10 grm. of indigo.

Further Experiments upon Acetic Acid Extraction.—The extraction of the indigo is accompanied by a certain loss of weight in the wool, and the extracted wool substance is not soluble in water, but is precipitated with the indigo, which accounts for the incorrect results obtained when such precipitated indigo is sulphonated and estimated by titration.

The wool substance is completely removed from the extracted indigo by means of boiling dilute sulphuric acid (20% by volume) without affecting the indigo, and this may be done by washing the filtered indigo upon a Gooch crucible, or glass filtering tube, using glass wool as the filtering medium. The wool substance is more quickly removed from the extracted indigo by washing with sulphuric acid as above, followed by boiling dilute ammonia (1:3), or boiling 10% sodium hydroxide, the preliminary treatment with sulphuric acid preventing the alkali from forming a colloidal solution of the indigo, which will pass through the filter. If gravimetric estimation is employed and the indigo is collected and weighed repeatedly in the same filtering tube, the indigo remaining in the tube serves as a filtering medium, and in this case the washing with boiling sulphuric acid can be omitted, the precipitate being washed directly with boiling ammonia or sodium hydroxide.

The following perfected method for analysis by acetic acid extraction has been finally adopted (Lloyd): The indigo is extracted at the boiling point in any of the forms of apparatus already described, using 3 to 15 grm. of material according to the percentage of indigo present. From 50 to 70 c.c

of glacial acetic acid are employed, and with the extractor A or B an additional quantity sufficient to fill the extraction tube and cause it to siphon or over-flow. At the end of the extraction the tube is left filled with the solvent. If the material is easily extracted, the boiling-flask may be heated over wire gauze, but when the time of extraction exceeds 2 hours the flask should be heated in an oil-bath. Under these conditions the amount of decomposition is not sufficient to affect the accuracy of the process for technical purposes.

The extract is allowed to stand until cold and is then filtered through a weighed glass tube containing quartz or glass wool, or upon a Gooch crucible. The indigo is washed twice with 10 c.c. of cold glacial acetic acid, then with 20 c.c. of boiling dilute acetic acid (30% by volume), and afterwards with water. To remove wool substance and colouring matters, the indigo is then well washed with boiling dilute sulphuric acid (20% by volume), the acid is removed by washing with water, and the indigo is then well washed with boiling ammonia (1:3) or with boiling 10% sodium hydroxide until the filtrate is no longer coloured. The alkali is now removed by washing with boiling water, then with a small quantity of acetic acid, again with boiling water, and finally with about 20 c.c. of alcohol. The filter is now dried at 110° and weighed. The washing with 20% sulphuric acid is only necessary when a new filter is used.

A slight modification of the above described method of procedure is em-ployed by Frank, who works in the following manner: The extraction is effected in the tube C, employing 70 to 100 c.c. of glacial acetic acid. The extract is allowed to cool, diluted with 100 c.c. of 50% alcohol, heated nearly to boiling and filtered hot through a Gooch crucible, containing coarse asbestos as filtering medium. These crucibles are readily pre-pared by keeping some asbestos soaking in 50% acetic acid and using as required. The collected indigo is washed with water, hot 2% sodium hy-droxide, boiling dilute hydrochloric acid (3 : 100), and finally with alcohol and ether. It is then dried for a short time in the water oven, put into a small beaker, and sulphonated at 70°–75° with 15–20 c.c. of pure conc. sulphuric acid for three-quarters of an hour. After cooling, it is poured into water, made up to 500 c.c., and titrated with $N/10$ permanganate, using 100 c.c. at a time, diluted with 200 c.c. of water. The indigo content is obtained from the factor 1 c.c. $N/10$ permanganate = 0.00146 grm. of indigo.

Experiments on the Estimation of Indigo by Extraction with Pyridine.— The powerful solvent action of pyridine upon indigo renders this liquid par-ticularly suitable for extracting indigo from the fibre, and superior in many respects to acetic acid.

The extraction may be carried out in any of the forms of extracting ap-paratus already described. When form C is used the material is made into a loose roll, or is wrapped in fine wire gauze, and put into the extraction tube; but felted, heavily milled, or tightly woven cloth is cut into thin strips. A little cotton wool is placed at the bottom of the tube to collect small fibres

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and to prevent the too rapid percolation of the condensed solvent, thus keeping the tube full of liquid.

To ascertain whether the extractions with pyridine were quantitative, and also to determine the most suitable working conditions, experiments were carried out by wrapping weighed quantities of indigo in wool, union, and cotton fabrics, then subjecting these to extraction, and weighing the indigo recovered. It was found that the wool was not attacked by pyridine, even upon long boiling, to as great an extent as occurs when acetic acid is used, but unless sufficient solvent is employed to keep the whole of the indigo in solution, heating of the boiling flask over a bare flame may cause a certain amount of decomposition of the indigo which crystallises on the sides of the flask. It was also observed that when a solution of indigo in pyridine is allowed to stand, a gradual disappearance of the colour occurs in the course of a few days, apparently through air oxidation, and it is therefore advisable to filter the extract without delay. If the extraction is effected in the siphon extracting tube, it is necessary to employ pyridine of fairly constant boiling point, but with the other forms of apparatus a good commercial pyridine may be used.

The wool substance is not dissolved to the same extent by pyridine as by acetic acid, and caustic soda completely removes the impurity from the precipitate.

That the wool is less attacked is also shown by the smaller loss of weight which it suffers, averaging 1.8% against 5.5 to 6% in extractions with acetic acid, after allowing in each case for the indigo present.

Indigo is far more soluble in cold pyridine than in cold glacial acetic acid, and the amount left in solution after cooling renders it necessary to precipitate the whole of the indigo by addition of a solvent miscible with pyridine in which indigo is insoluble.

When a pure indigo-dyed cloth was extracted with 100 c.c. of pyridine, filtered off after 1 hour's standing on ice, and the indigo present in the blue filtrate neglected, the percentage found was 1.23. On the other hand, 1.28% of indigo was obtained when the filtrate was concentrated and diluted with 50% alcohol.

Iron lakes of tannin materials are fairly easily decomposed by pyridine, and when the wool has been heavily loaded with iron, some difficulty may be encountered owing to precipitation in the boiling flask. The precipitated indigo may also require a more protracted washing with acid and alkali.

But although the direct extraction of such materials may yield slightly high results, the previous removal of the topping dyestuff and iron is not advisable and may even give rise to a small loss of indigo.

The use of pyridine has especial advantages in the analysis of thick, felted, heavily milled or hard spun twill materials, which are very troublesome and difficult to extract completely by means of acetic acid.

It also has the advantage of leaving the wool in a better condition than does acetic acid.

The following table (Lloyd) gives the limits of accuracy in the various methods of analysis which have been examined:

	Possible error. (Percentage upon total indigo)
I. Rawson's hydrosulphite method................	0 to −13
II. Brylinski's method.........................	+30.0 to +44.7
III. Acetic acid extraction followed by after-treatment with caustic soda..........................	− 1.7 to − 2.8
IV. Möhlau and Zimmermann's method............	− 3.8 to −11.0
V. Acetic acid extraction followed by washing with 20% sulphuric acid and caustic soda or ammonia	− 0.5 to + 3.1
VI. Preceding method when other dyestuffs are present	− 1.3 to + 3.1
VII. Pyridine extraction without after-treatment of precipitate........................	+ 8.7 to +30.0
VIII. Pyridine extraction followed by washing with 20% sulphuric acid and caustic soda or ammonia....	− 2.2 to − 4.0
IX. Preceding method when other dyestuffs are present	− 3.4 to + 1.5

The following method of procedure has been finally adopted for **analysis by pyridine**: The extraction tube (usually form C is preferred) is charged with from 3 to 15 grm. of material (viz., sufficient to give about 0.05 grm. of indigo), the hole at the bottom of the tube being covered with a little cotton wool. Cloth is made into a loose roll or is enclosed in thin wire gauze. 100 c.c. of commercial pyridine (b. p. 110°–127°) are put into the boiling flask, which is heated over wire gauze or upon an air-bath. Either a water condenser, or simply a long air condenser, is employed. The extraction is continued until the pyridine runs through quite colourless, which usually requires from 2 to 3 hours. The extract is then distilled down to about 20 or 30 c.c., the pyridine recovered being kept for future extractions. The extraction flask is then set aside to cool, when the greater part of the indigo separates in well-formed bronzy crystals. To complete the precipitation of the indigo, 150 c.c. of 50% alcohol are added, and after heating to boiling, the liquid is filtered either through an ordinary Gooch crucible prepared with filter paper or asbestos, or through a glass filtering tube containing glass wool.

Before weighing the filter for use, it is washed with exactly the same liquids as are to be used for washing the precipitated indigo and then dried at 110°. The filtration through a Gooch crucible is very rapid, taking less than 2 minutes. The precipitate is washed on the filter successively with hot 50% alcohol, hot 2% caustic soda, hot dilute hydrochloric acid (3 : 100), hot water, alcohol, and finally ether. The crucible is then dried at 110° and weighed. Lloyd prefers to wash with 20% sulphuric acid and then with hot 10% caustic soda or ammonia, then with 20 c.c. of glacial acetic acid, finally with water and with alcohol. The appearance of the indigo precipitate is a guide to its purity. It should form a bronzy crystalline powder, which, when analysed by the Bloxam-Perkin method,[1] tests 100%.

[1] See Vol. V, p. 394.

A dull appearance shows the presence of impurities. In order to shorten the operation it is advisable after about ¾ of an hour's extraction to remove and repack the material in the tube, placing what was formerly inside in the outside position. Heavy materials should be cut into fine strips and also repacked after some time. If it is desired to estimate the indigo by titration instead of by direct weighing, the precipitate is collected on a Gooch crucible, the bottom of which is covered with a little asbestos. After washing with acid and alkali as above, and drying for a short time, the crucible is placed in a small beaker containing 15 to 20 c.c. of pure concentrated sulphuric acid, and the indigo is sulphonated by heating in an oven to 70°–80° for 45 minutes. The solution is then made up to 500 c.c. and titrated with $N/50$ permanganate, using 100 c.c. at a time, diluted with 200 c.c. of water. The percentage of indigo is found from the factor 1 c.c. $N/50$ permanganate = 0.00146 grm. indigo (Frank), or = 0.00147 grm. (Lloyd), obtained by sulphonating pure sublimed indigo and titrating in the same way. This factor is more exact than that employed by Rawson, viz., 0.00150.

In treating cloths which have been heavily topped with iron and tannin materials, Lloyd thinks it advisable to remove as far as possible these lakes, prior to the pyridine extraction, by alternate boiling with a solution containing 5% oxalic acid, and with dilute ammonia (5 : 100), until colour is no longer extracted. Frank, however, considers that this previous treatment of the dyed cloth is unnecessary, provided the extracted indigo is efficiently washed with acid and alkali as described above.

Analysis of Commercial Indigo-dyed Materials by the Pyridine and Acetic Acid Methods.—Both the acetic acid and pyridine methods are capable of giving accurate results under the conditions laid down.

In order to subject these methods of analysis to a rigid test, and at the same time to effect a comparsion between them, analyses were carried out upon a large number of materials dyed under practical conditions, the results of which are given in the tables which follow. The first series represents a range of pure indigo shades, from a light blue to a very dark navy. The second series consists of a medium shade of indigo-dyed cloth which was afterwards topped with a variety of acid and mordant colouring matters. In the third series the wool was bottomed with various colouring matters and afterwards dyed in the indigo vat. The colouring matters selected as topping or bottoming colours were those most likely to be employed in practice. The fourth series consists of a variety of commercial indigo and navy blue cloths of different makes. The fifth series contains a number of official and Government cloths. In series six the same dyestuffs are employed both as bottoming and topping colours, in order to ascertain whether the total tintometric value of the mixed shade is thereby affected.

The analyses were made gravimetrically with pyridine and both gravimetrically and volumetrically with acetic acid.

SERIES I.

Reference number	Description	Percentage indigo found		
		Acetic acid or other solvent[1] by weight (Lloyd)	Acetic acid by titration with KMnO₄ (Frank)	Pyridine by weight (Frank)
		%	%	%
1	Pure indigo........	0.42	0.54	0.44
2	Pure indigo........	0.51	0.66	0.54
3	Pure indigo........	0.71	0.75	0.64
4	Pure indigo........	0.85	0.92	0.80
5	Pure indigo........	1.14	1.08	1.05
6	Pure indigo........	1.30	1.29	1.25
7	Pure indigo........	1.46	1.44	1.33
8	Pure indigo........	1.68	1.71	1.70
9	Pure indigo........	2.13	2.20	2.27
10	Pure indigo........	2.43	2.50	2.50
59	Pure indigo........	2.23	2.10	2.10
60	Pure indigo........	2.47	2.41	2.35
61	Pure indigo........	2.72	2.95	2.71
62	Pure indigo........	3.69	3.54	3.42
63	Pure indigo........	4.57	4.43	4.42

SERIES II. (TOPPED BLUES.)

Reference number	Description	Percentage of indigo found		
		Acetic acid or other solvent[1] by weight (Lloyd)	Acetic acid by titration with KMnO₄ (Frank)	Pyridine by weight (Frank)
		%	%	%
13	Standard Indigo, bottomed with 2% chrome alone....	1.68	1.79	1.73
	Standard Indigo, topped with:			
14	2% bichromate and 2% Sulphon Cyanine 5R extra..	1.69	1.72	1.72
15	2% bichromate and 1½% Brilliant Aliz. Blue R pdr..	1.71	1.74	1.67
16	1½% Topping Violet RTN (B.A.S.F.).............	1.67	1.76	1.66
17	2% Indocyanine 2R (Ber. Co.)....................	1.68	....	1.72
18	1½% Erio Fast Purple A (Geigy)...	1.69	1.77	1.69
19	2% Fast Acid Violet R (M. L. & B.)...............	1.71	1.75	1.72
20	1¼% bichromate and 2% Chrome Blue A (B.A.S.F.).	1.64	1.73	1.70
21	1¼% bichromate and 2% Palatine Chrome Blue B...	1.59	1.78	1.77
22	2% bichromate and 2% Hæmatine crystals.........	1.70	1.68	1.70
23	1¼% bichromate and 2% Eriochrome Azurol B.....	1.65	1.72	1.68
24	1¼% bichromate and 2% Omega Chrome Cyanine B.	1.68	1.67	1.62
25	2% bichromate and 1¼% Alizarin Blue B..........	1.71	1.68	1.69
26	2% bichromate and 8% Alizarin Blue GW double...	1.67	....	1.65
27	2% bichromate and 2% Sulphon Dark Blue 2B.....	1.72	1.69	1.72
28	2% bichromate and 2% Wool Fast Blue BL (By)...	1.67	1.74	1.62
29	2% bichromate and 2% Sulphon Cyanine GR extra.	1.69	1.69	1.65
30	2% bichromate and 1½% Indochromine 2R conc....	1.73	1.69	1.69
31	2% bichromate and 20% Cudbear.................	1.70	1.65	1.65
39	2% bichromate and 7% Gallein paste.............	1.67	1.74	1.66
40	2% bichromate and 7% Gallocyanin paste..........	1.74	1.73	1.62
41	2% bichromate and 5% Alizarin Cyanin 3R double paste.	1.69	1.73	1.60
42	2% bichromate and 2% Lanacyl Violet B (Cass.)....	1.65	1.76	1.69
43	2% bichromate and 2% Soluble Blue (L. D. Co.)....	1.69	1.73	1.68
44	2% bichromate and 2% Acid Chrome Blue 2R (By.).	1.65	1.75	1.65
45	2% bichromate and 2% Eriochrome Blue BR (Gy.)..	1.67	1.74	1.62
46	1% Acid Violet 4BRS (Sandoz).....................	1.70	1.76	1.72
47	1¼% Omega Light Violet R (Sandoz)..............	1.72	1.75	1.72
50	Myrobolans and "nitrate of iron"....................	1.71	1.73	1.66
55	3% bichromate and 3½% Fustic...................	1.73	1.72	1.64
56	2% Picric Acid....................................	1.70	1.76	1.68
57	2% bichromate and Logwood......................	1.72	1.70	1.62
58	Myrobolans and ferrous sulphate..................	1.72	1.65	1.63
83	Indigo extract....................................	1.66	....	1.66
	Standard Indigo filled with:			
85	Starch..	1.62	1.60	1.62
86	Magnesium chloride..............................	1.61	....	1.62

[1] Some of these figures are the average of several obtained with acetic acid, pyridine, and piperidine

SERIES III. (BOTTOMED BLUES.)

Reference number	Description		Percentage of indigo found		
			Acetic acid or other solvent[1] by weight (Lloyd)	Acetic acid by titration with KMnO₄ (Frank)	Pyridine by weight (Frank)
	Bottomed with:		%	%	%
32	1 % Aso Fuchsine G.....................	All dyed together in same indigo vat.	2.20	2.37	2.14
33	2 % bichromate and 8 % Cudbear.........		2.16	2.02	1.97
34	2 % bichromate and 1 % Aliz. Red IWS (M)		2.09	2.07	1.94
35	2 % bichromate and 20 % Sanderswood.....		2.13	2.00	1.98
36	2 % bichromate and 30 % Camwood........		2.23	2.05	2.02
37	2 % bichromate and 20 % Barwood.........		2.14	2.05	1.96
38	1 % bichromate and 1 % Omega Chrome Red B.		2.20	2.13	2.08

SERIES IV. (BLUES ON VARIOUS MATERIALS.)

Reference number	Description		Percentage of indigo found		
			Acetic acid or other solvent[1] by weight (Lloyd)	Acetic acid by titration (Frank)	Pyridine by weight (Frank)
			%	%	%
64	Pure indigo on 2-ply worsted	Dyed together in same vat.	1.64	1.65	1.58
65	Pure indigo on fine woollen		1.98	1.97	1.89
66	Pure indigo on fine merino....		2.30	2.40	2.28
67	Pure indigo on coarse worsted		1.61	1.78	1.54
68	Lighter shade of No. 64.......	Dyed together in same vat.	0.51	0.49	0.48
69	Lighter shade of No. 65		0.70	0.54	0.55
70	Lighter shade of No. 66		0.84	0.73	0.75
71	Lighter shade of No. 67		0.69	0.54	0.54
84	Indigo on cotton warp material....................		1.72	....	1.63
51	A commercial indigo.............................		0.98	0.71	0.71
52	Another commercial indigo........................		1.03	0.70	0.65
53	No. 1 Navy blue................................		3.86	3.76	3.80
54	"Pure" indigo on worsted cloth (red bottom)........		3.44	....	3.41
12	No. 2 navy blue on grey serge.....................		2.38	....	2.32

SERIES V. (OFFICIAL AND GOVERNMENT STANDARDS.)

Reference number	Description	Percentage of indigo found by extraction with pyridine (by weight)	
		(Lloyd)[2]	(Frank)
		%	%
72	No. 1 customs......................	2.52	2.35
73	No. 2 custom worsted..............	2.59	2.54
74	No. 3 blue.........................	4.18	4.18
75	(Old) Pantaloon cloth..............	3.35	3.23
76	Post-office pilot...................	3.12	3.22
77	4A blue...........................	3.25	3.0
78	Box cloth.........................	4.20	4.23
79	4B blue...........................	2.60	2.54
80	(Old) Metropolitan Police blue.....................	2.90	2.82
81	No. 2 artillery....................................	3.04	3.11
82	Navy tartan......................................	3.03	2.91
87	Metropolitan Police, blue greatcoat................	2.46	2.50

[1] Some of these figures are the average of several obtained with acetic acid, pyridine, and piperidine.
[2] In some cases analyses were made with piperidine as well as with pyridine.

SERIES VI. (EFFECT OF TOPPING OR BOTTOMING ON TOTAL SHADE.)

Reference number	Description	Percentage of indigo found	
		Acetic acid or other solvent[1] by weight (Lloyd)	Pyridine by weight (Frank)
		%	%
109	White cloth dyed with Indigo....................	1.36	1.34
	Bottomed with		
110	9₄% bichromate and 1% Eriochrome Red BR....	1.54	1.56
111	1% Eriochrome Red BR without chrome.......	1.53	1.54
112	2% bichromate and 1% Alizarin Red IWS......	1.32	1.34
113	Previously chromed wool (2% chrome) dyed with Indigo.	1.37	1.47
	Indigo, No. 109, topped with		
114	1% Eriochrome Red BR and 9₄% bichromate..........	1.35	1.36
115	1% Eriochrome Red BR without bichromate............	1.33	1.36
116	1% Alizarin Red IWS and 2% bichromate.............	1.26	1.41
	Indigo, No. 113, topped with		
117	1% Eriochrome Red BR and 9₄% bichromate..........	1.37	1.48
118	1% Eriochrome Red BR without bichromate...........	1.36	1.48
119	1% Alizarin Red IWS and 2% bichromate.............	1.24	1.46

(Between rows 110–113 appears the bracketed note: "All dyed at same time in indigo vat.")

III. Determination of Percentage Colour Effect Due to Indigo.—It is obvious that the simple statement of the percentage of indigo upon a cloth, as found by analysis, does not afford to anybody but an expert an idea of the quality of the dye. What is required by manufacturers, merchants, and the public is a means of knowing what proportion of the total depth of colour is due to the indigo present.   The solution of this problem is a difficult one, as besides the difficulty of finding an instrument capable of accurately measuring depth of colour on fabrics, the dyestuffs accompanying the indigo are not necessarily blue, but are frequently violet or red.   A solution has been attempted by employing the Lovibond tintometer as the colour-measuring instrument.   Since the relative proportions of red, yellow, and blue will vary in different shades, the measure of depth must be taken as the total number of colour units obtained by adding together the units of red, yellow, and blue, given by the glasses required to match the pattern.   By applying this instrument to the series of shades of pure indigo, dyed on white wool, of which the analyses are given in the table Series I, it has been found that the depths of shade thus expressed lie upon a regular curve (see Fig. 2, p. 440).   There is thus a definite relation between the percentage of indigo on the material by weight and the tintometric reading.   The tintometer readings from which this curve is constructed are given in the following table.   These readings were obtained in a north light between 10 a. m. and 12 a. m. on February 18, 1914, a bright morning with light blue sky and white clouds:

[1] Some of these figures are the average of several experiments with different solvents.

Reference number	Percentage of indigo present (by analysis). (Mean result)	Tintometer measurements			
		Red	Yellow	Blue	Total units
1	0.45	4.0	2.6	10.9	17.5
2	0.55	4.9	2.9	11.4	19.2
3	0.70	6.1	3.4	12.1	21.6
4	0.85	7.1	3.5	12.7	23.3
5	1.05	8.4	3.5	12.96	24.86
6	1.25	9.0	4.0	13.0	26.0
7	1.40	9.6	4.3	13.1	27.0
8	1.70	10.8	4.7	13.5	29.2
9	2.20	11.7	5.9	13.6	31.2
10	2.50	12.2	6.6	13.7	32.5
61	2.71	12.5	8.3	13.0	33.8
62	3.55	13.2	9.4	14.0	36.6
63	4.45	13.7	9.8	14.5	38.0
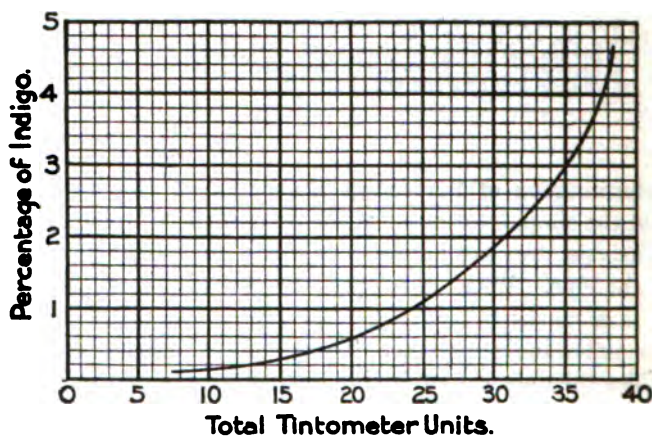
FIG. 16.

By employment of such a curve it is possible (assuming its accuracy) to determine from the tintometric readings of a given cloth, the percentage of indigo it contains if dyed with indigo only; or when dyed with other colours in addition, the percentage of indigo which would be required to give a shade of corresponding depth if indigo alone was used. Furthermore, in this case it is possible when the actual percentage of pure indigo on the cloth, as determined by analysis, is known, to express the colour effect due to the latter (obtained from the curve) as a percentage of the total colour, measured in terms of the amount of indigo which would be required to give the total depth of shade of the pattern if indigo alone was used. This ratio, termed the "percentage colour effect due to indigo," is given by the expression:

$$x = \frac{A \times 100}{C}$$

in which A = the percentage of indigo found by analysis and C = percentage of indigo given by the curve corresponding to the total tintometer units of the

pattern. This value cannot be obtained from the direct ratio between the tintometer colour units corresponding to the indigo present and the total shade units, since the depth of shade is not directly proportional to the amount of dyestuffs present, but increases at a constantly diminishing rate as the shade becomes darker. It has been ascertained by examination of a large number of patterns dyed with pure indigo (Series IV) that the same percentage of indigo gives substantially the same tintometric reading for all classes of material composed of wool independently of the quality and weave. The variation observed is certainly less than 1 in 20, the tintometric reading being slightly higher for thick materials than for thin. It has also been established that in mixed shades the tintometric reading is the same whether the additional dyestuff has been employed as a bottoming or as a topping colour (see Series VI).

Experiments made with this method have shown that for blues of light and medium depth (up to, say, 2.5% of indigo) results of sufficient exactitude for commercial purposes can be obtained, but that for heavy shades which approach black the tintometer is incapable of measuring colour depth with sufficient accuracy, since with dark shades a relatively large increase in the percentage of indigo produces only a small increase in the tintometric reading.

Standard conditions for reading the tintometer cannot be laid down, as somewhat different readings are obtained for the same pattern on different days on account of variations in daylight. The readings of different observers may also vary on account of differences of light, eyesight, and instrument. It is therefore recommended that in applying this method the curve given above should only be used as an approximation and should be corrected at the time of use by taking tintometer readings of a series of four or five pure indigo shades kept as standards, in which the percentages of indigo have been accurately determined by analysis. Having also obtained by analysis the percentage of indigo in the material under examination, the value for the "percentage colour effect due to indigo" can then be deduced from the corrected curve. For example, a sample of cloth dyed with indigo and topped with other dyestuffs is found to contain 1.5% of indigo by analysis, and gives a total reading of 32.5 colour units on Lovibond's tintometer. From the curve it is seen that a shade dyed with pure indigo to give a reading of 32.5 colour units has to contain 2.4% of indigo. Therefore, the "percentage colour effect due to indigo" on the pattern is:

$$\frac{1.5 \times 100}{2.4} = 62.5$$

All the analyses and tintometer measurements are made upon cloth containing its "condition moisture," usually about 14%.

For the investigation of heavy shades, with which the above method of deducing the "percentage colour effect due to indigo" is unreliable, another

method is available which, so far as the experiments go, appears to be capable of giving satisfactory results with all depths of shade. This consists in entirely removing the indigo from the pattern by extraction with a suitable solvent and measuring the depth of the residual colour (dyestuff used for topping or bottoming) in total colour units by the Lovibond tintometer. The indigo equivalent of this residual colour (= B) is then found from the curve, and knowing the actual percentage of indigo present on the cloth, as determined by gravimetric analysis (= A), the "percentage colour effect due to indigo" is given by the equation:

$$x = \frac{100\,A}{A + B}$$

In order to test this method of procedure and to compare it with the previous one, five navy blue serges were tested by the tintometer, together with the bottoming dyes (mainly Alizarin Reds) upon which they were dyed, and also the same serge dyed simultaneously on a white bottom in the same vat, and therefore containing approximately the same quantity of indigo. In each case the total number of tintometer units found were translated by means of the curve into percentage indigo equivalents, the following numbers being obtained:

Number of sample	Indigo equivalents from curve (percentages)		
	Blue dyed on white serge	Red bottom (=B)	Compound shade (=C)
1	2.41	0.38	2.84
2	1.55	0.48	2.10
3	1.35	0.50	1.85
4	1.46	0.78	2.30
5	1.96	1.25	3.25

The actual indigo present on the patterns was found by analysis to be as follows:

Number of sample	Percentage indigo present		
	Indigo on white serge	Indigo Compound shade	Average (=A)
1	2.39	2.29	2.34
2	1.52	1.52	1.52
3	1.28	1.33	1.31
4	1.44	1.41	1.42
5	1.85	1.88	1.86

Calculating for these five patterns the "percentage colour effect due to indigo," firstly from the tintometer readings alone, and secondly by making use of the two equations:

$$\text{I. } x = \frac{100\,A}{C} \qquad \text{II. } x = \frac{100\,A}{A + B}$$

the following results are obtained:

Number of sample	Percentage colour effect due to indigo		
	From tintometer values alone	By method I	By method II
1	82.2	84.8	86.0
2	72.4	73.8	76.0
3	70.8	72.9	72.4
4	61.7	63.5	64.5
5	57.2	60.3	59.8

The results by the different methods are therefore in good accord.

To be in a position to make use of the second method it is necessary to be able to strip the indigo completely from a compound shade and to leave the topping or bottoming colour practically unaltered. A small *change* of shade of the latter does not matter, provided the *depth* is not affected. In a large number of cases the stripping may be satisfactorily done by employing either boiling glacial acetic acid or boiling pyridine as the stripping agent, for whereas many dyes are removed from wool by one or the other of these solvents, comparatively few of them are stripped by both, those which are dissolved by the one being usually not affected by the other. In using these solvents care should be taken that they are anhydrous, as a small percentage of water increases their solvent action on dyestuffs other than indigo. The acetic acid should therefore be frozen and the separated crystals remelted, whilst the pyridine should be carefully dried over solid sodium hydroxide. For the same reason, the pattern to be extracted should be previously dried in a steam oven.

As there are a few colouring matters which are removed from the wool by both acetic acid and by pyridine, some other solvent is occasionally necessary. Benzaldehyde, which is a very powerful solvent for indigo, can be employed with good effect in many cases, especially for the quick and complete removal of indigo prior to making a qualitative examination of the topping or bottoming colour. Owing, however, to the tendency possessed by benzaldehyde to form condensation products with indigo which give a yellow tint to the wool, it is unsuitable for stripping when it is required to make tintometric estimations of the residual colour. For the latter purpose, the best extracting agent is cresol mixed with a certain proportion of a hydrocarbon of lower boiling point. 100 parts cresol (best commercial cresylic acid, 97–98%) with 30 parts of "solvent naphtha" of boiling point 125°–140°, or 75 parts of cresol with 25 parts of "turpentine substitute" or heavy petrol of boiling point 155°–170° have proved to be suitable mixtures. The extraction is effected in an *ordinary* Soxhlet extractor, which is provided with an air or water condenser. The sample, previously dried in a steam oven, is laid on a little loose wool and covered lightly with a further layer of loose wool, a thermometer being placed with its bulb in contact with the pattern. The proportion of hydrocarbon to cresol is so adjusted that the temperature of the extracting liquid round the pattern

does not exceed 110°, and is preferably about 100°–105°. The lower the temperature at which the extraction of the indigo can be effected, the less the danger of disturbing the concomitant dyestuffs. Care should be taken not to continue the extraction after the indigo has been completely removed or a loss of residual colour may occur. Carried out with care this method of separation seems capable of almost universal application, and the only cases in which the original depth of the bottoming or topping colour was not obtained were with Soluble Blues, Picric Acid, and redwoods, in which there was strong evidence that the bottom dye had already been partly removed in the indigo vat.

The behaviour of the four solvents selected towards a variety of colouring matters dyed upon wool in conjunction with indigo is shown in the following table. The minus sign indicates that the colour is stripped, the plus sign that it is not affected or only slightly, and the sign (+ −) that it is partially removed. Those dyes marked by an asterisk are changed in shade to violet.

Apart from the above-described use of extraction methods for estimating

Name of colouring matter	Acetic acid	Pyridine	Benzaldehyde	Cresol mixture
Sulphoncyanine 5R extra and GR (By.)	−	−	+	+
Brilliant Alizarin Blue R (By.)	+−	+−	+	+
Topping Violet RTN (B.A.S.F.)	+	−	+	+
Indocyanine 2R (Ber.)	+	+	+	+
Erio Fast Purple A (Gy.)	+	−	+	+
Fast Acid Violet R (M.L. & B.)	+−	+−	+	+
Chrome Blue A (B.A.S.F.)	+	+	+	+
Palatine Chrome Blue 2B (B.A.S.F.)	+	+	+	+−
Hæmatine crystals	−	+	+	+−
Eriochrome Asurol B (Gy.)	−	+	+	+−
Omega Chrome Cyanine B (Sz.)	+	+	+	+−
Alizarin Blue Black B (By.)	+−	+	+	+
Alizarin Blue SW (B.A.S.F.)	+−	+	+	+
Sulphon Dark Blue 2B	−	−	+	+
Wool Fast Blue BL (By.)	+•	−	+	+−
Indochromine 2R conc. (Sz.)	+•	+	+	+−
Cudbear on bichromate	−	−	+−	+
Azofuchsine	−	−	+	+
Alizarin Red IWS (M.L. & B.)	+−	+	+	+
Sanderswood and bichromate	−	+−	+−	+−
Camwood and bichromate	−	+−	+−	+−
Barwood and bichromate	−	+−	+−	+−
Omega Chrome Red B	+	−	+	+
Gallein paste	−	+	+	−
Gallocyanin	−	+	+−	+
Alizarin Cyanine 3R	−	+	+	+
Lanacyl Violet B (Cass.)	+	−	+	+
Soluble Blue	−	−	−	+−
Acid Chrome Blue 2R (By.)	+•	+−	+	+
Eriochrome Blue BR (Gy.)	+	−	+	+
Acid Violet 4BRS (Sz.)	+	−	+	+
Omega Light Violet R (Sz.)	+	−	+	+
Myrabolans and iron	−	+	−	+
Fustic and bichromate	−	+	+	+
Picric acid	−	+	+	+−
Logwood and bichromate	−	+	+	+
Indigo extract	+	−	+	+
Eriochrome Red BR (Gy.)	+	−	+	+

quantitatively the relative proportion of the colour depth which is due to indigo, they afford a ready means of roughly gauging the amount of the topping or bottoming colour, and of testing it for fastness, which should be of great service to the merchant and dyer. Moreover, after the removal of the indigo, the concomitant dyestuff or dyestuffs can be easily identified by making use of a scheme of qualitative analysis such as that of Green, for although the identification of other dyestuffs in presence of indigo is a difficult and often impossible task, this becomes a fairly straightforward matter when the indigo has been removed. Thus, for example, in investigating a navy blue cloth supplied to a London railway company, it was easily shown that it consisted of indigo topped with a mixture of Eriochrome Azurol and Logwood. In cases in which more than one topping or bottoming colour has been employed, an indication may often be obtained by comparing the residual colours left by different stripping agents. The testing of the residual colour for fastness to washing, light, etc., may frequently be an important factor in judging the quality of a navy blue shade, as it is obvious that the fastness of the concomitant dyestuff should also be taken into account together with the percentage of indigo present.

In order to submit to further test the general applicability of the stripping method for the quantitative estimation of the "percentage colour effect due to indigo," a number of official and Government cloths (Series V in foregoing tables) were subjected to independent examination by two observers, one of whom (Frank) employed cresol mixture, whilst the other (Lloyd) used dry pyridine for removing the indigo. In each case the tintometric readings of the stripped patterns were all observed together, and the units of total colour converted into indigo equivalents by reference to a curve which was constructed at the time. The results are shown in the following table:

Number of sample	Indigo found by analysis (=A)		Indigo equivalent of residual colour (=B)		Percentage colour-effect due to indigo $\left(=\frac{100\,A}{A+B}\right)$	
	Frank	Lloyd	Frank	Lloyd	Frank	Lloyd
72	2.35	2.52	0.55	0.72	80	77
73	2.54	2.59	0.20	0.22	92	92
74	4.18	4.18	0.68	0.72	86	85
75	3.23	3.35	0.68	0.55	83	86
76	3.23	3.12	0.30	0.36	91	90
77	3.0	3.25	0.65	0.65	82	83
78	4.23	4.20	0.30	0.32	93	93
79	2.54	2.60	0.75	0.48	77	84
80	2.82	2.90	0.60	0.62	81	82
82	2.91	3.03	0.40	0.32	88	90

It appears from these results that the maximum error of the process is about 7% and with practice would not exceed 5%.

A few observations are desirable with regard to the use of the tintometer. The best form of this is the "three aperture" instrument, with which the

pattern can be placed in the central opening whilst matching glasses are used on either side of it.   With this form of instrument it is easy to confirm the results arrived at, by varying the matching glasses on one side, and differences in depth of shade are more apparent than with the duplex tube.   The readings should be made with a good north light coming from the front, and the instrument should be placed upright or at an angle not less than 70° to the horizontal, taking care, however, that there is no top light to cast a shadow on the pattern.

The following additional observations have been made in the course of the above work:

(1) If ordinary wool and chromed wool are dyed together in a vat, practically the same percentage of indigo is absorbed by each.

(2) Although the chroming of indigo dyed wool generally causes some loss of indigo, the after-chroming of a topping dyestuff does not appreciably affect the indigo unless the temperature or amount of chrome is too high.

(3) Many dyestuffs used for bottoming purposes, whether acid or chrome colours, cause the wool to absorb more indigo from the vat than does untreated wool under the same conditions.

(4) There is no difference in the final tintometric value when, for example, a red colour is topped on indigo dyed material, or the indigo topped on the red dyed material, provided that the amount of indigo and red colouring matter are the same in each case.

Analysis of Indigo Containing Starch.—W. Thomson[1] states that Rawson's permanganate process is not applicable for the direct estimation of indigo containing starch, and suggests that such samples be previously heated to 90° with a 4% solution of hydrochloric acid, filtered and washed with hot water and dried before sulphonation.

These observations were corroborated by Frank and Perkin.[2]

ERRATA IN VOL. V.

Page 116, line 7 from bottom, insert comma after "dyeing." Line 8 from bottom, italicise "colour bases." Line 4 from bottom, for "unsoluble" read "insoluble."

Page 117, line 16 from top, italicise "neutral dyestuffs."

Page 119, line 6 from bottom, for "513" read "435."

Page 120, line 6 from top, for "indamines" read "indamine."

Page 124, line 21 from top, between "and" and "much" insert "is."

Page 131, line 8 from top, for "Dinitroresorcnol" read "Dinitroresorcinol."

Page 131, last line, for "dilutiou" read "dilution."

Page 135, 2 lines from bottom, for "Chyrsoln" read "Chrysoln."

Page 135, line 9 from top, replace "and" by "only."

Page 136, line 9 from top, for "wth" read "with."

Page 137, second footnote, for "Polyp" read "Polyt."

Page 138, space 3 from bottom, replace "Ocange" by "Orange."

[1] *J. Soc. Dy. and Col.*, 1911, 49.
[2] *J. Soc. Chem. Ind.*, 1912, 372.

Page 140, line 2 from bottom, for "Ponccau" read "Ponceau."

Page 147, line 13 from top, for "diamine nitrazole" read "diaminogen."

Page 148, space 5 from top, delete "R salt." Space 7 from top, for "from G salt. Isomers from R salt" read "From R salt. Isomers from G salt."

Page 148, space 10 from top, for "R salt" read "β-naphtholsulphonic acid S."

Page 149, space 4 from bottom, for "phenatidine" read "phenetidine."

Page 152, space 8 from bottom, for "trisulphonic" read "β-naphtholtrisulphonic."

Page 160, first line, after "solution" insert comma.

Page 164, lines 18 and 19 from top, for "salt R" read "R salt."

Page 165, lines 1 and 2 from top, formula should be "$C_{10}H_5(NaSO_3)_2.N_2.C_{10}H_4.N_2.-C_{10}H_5$."

Page 165, line 13 from top, for "β-naphthol-γ-disulphonic acid" read "β-naphthol-disulphonic acid G."

Page 176, line 5 from top, for "G" read "K."

Page 178, space 4 from bottom, for "From sulphanilic and naphthionic acid" read "From m-aminobenzenesulphonic and naphthionic acids."

Page 180, space 3 from bottom, formula for Benzo-grey should be

$$C_6H_4 - N = N - (2)C_6H_3 \begin{cases} (1)OH \\ (4)CO_2Na \end{cases}$$
$$C_6H_4 - N = N - (4)C_{10}H_6(1) - N = N - (2)C_{10}H_5 \begin{cases} (1)\ OH \\ (4)\ SO_3Na. \end{cases}$$

Page 186, third space from bottom, formula for Rouge M, for "$C_{10}H_4$" read "$C_{10}H_5$."

Page 198, space 4 from bottom, for "From γ acid" read "From D acid."

Page 202, replace "the last named dye" by "Benzopurpurin 4B."

Page 203, line 9 from top, for "α-naphtholdisulphonic acid" read "β-naphtholdisulphonic acid."

Page 203, line 15 from bottom, for "2" read "two." Line 11 from bottom, for "1" read "one."

Page 204, line 14 from top, for "or stannous" read "of stannous."

Page 204, line 14 from bottom, for "$C_{10}H_5(NH_2)OH$" read "$C_{10}H_6(NH_2)OH$."

Page 206, first space at top, "$C_6H_4:(N_2H_2)$" should be "$C_6H_4:(NH_2)_2$."

Page 207, line 8 from top, for "Leibermann" read "Liebermann."

Page 208, line 19 from top, for "-acetal-" read "-acetyl-."

Page 213, line 4 from bottom, between "and" and "used" insert "when."

Page 215, line 10 (heading), omit full stop after "Paste."

Page 216, table in centre of page belongs to footnote which it should follow.

Page 226, line 12 from top, for "mordan" read "mordant."

Page 442, line 16 from bottom, for "simple" read "similar."

Page 444, line 16 from top, for "being" read "is."

Page 448, line 26 from top, for "becomes coloured dilution" read "becomes coloured on dilution."

Page 451, L. H. S. third line, for "saffranine" read "safranine."

Page 452, L. H. S. third line, for "saffranine" read "safranine."

Page 453, L. H. S. at bottom, delete "(cerumn)."

Page 453, line 6 from bottom, for "turn to a yellow" read "turn it yellow."

Page 454, right-hand bottom corner, in both cases for "saffranine" read "safranine."

Page 464, line 9 from bottom, for "Is" read "If."

Page 476, line 14 from top, for "value" read "loss," after which insert semicolon.

Page 480, first line, after "Blue" insert "is."

Page 487, line 1, delete comma after "spirit."

Page 488, line 17 from bottom, after "dyed" insert "with."

Page 490, italicise names of reagents.

Page 516, line 1, for "OR VEGETABLE" read "ON VEGETABLE."

Page 543, line 7, for "HNO$_{12}$" read "HNO$_2$."

Page 569, second and third spaces, for "Fbre" read "Fibre."

Page 573, second space, for "Decolurised" read "Decolourised" and for "Substanive" read "Substantive."

Page 574, third space, for "Diamined" read "Diamine."

Page 592, heading of column, for "Soluton" read "Solution."

Page 598, space 10, for "Sapphiroll" read "Sapphirol."

Page 611, sixth space, delete "continued."

Page 615, space 3, column 10, for "become" read "becomes."

COLOURING MATTERS IN FOODS.

By ALBERT F. SEEKER, B.S.

Since the publication of Vol. V of the present edition the efforts of food analysts have been directed mainly towards isolating the colours from food in a condition to permit of their identification, this being a result of the tendency of governmental regulations to tolerate the use of certain colours provided they are harmless and do not conceal inferiority or otherwise promote fraud. In a comprehensive work "*Coal-tar Colours Used in Food Products.*" B. C. Hesse[1] has discussed the subject from a commercial, scientific and administrative standpoint, giving a list of the dyes that have been used, the commercial requirements of such colours, a compilation of records and work concerning the physiological effect of the dyes, legal enactments in various countries and recommendations by associations and individuals as to their use, and requirements concerning the degree of cleanliness and purity of the dyes to be employed for this purpose, together with methods for their analysis, the methods given being applicable only to the dyes themselves.

The limitation of the number of dyes permitted in food has resulted to a certain extent in increasing the use of mixtures of dyes to produce the desired shades and for this reason it has become of the greatest importance to modify analytical procedure so that such mixtures may be recognised when present and the individual colours properly separated and identified. An admirable systematic procedure to attain this end has been devised by W. E. Mathewson for the A. O. A. C.[2] In this method the basic dyes are first removed from alkaline solution by shaking with ether, the aqueous mixture being then strongly acidified and shaken with amyl alcohol to remove most of the acid colours. Light Green S. F. Yellowish and similar strongly sulphonated triphenylmethane dyes which may still remain in the aqueous liquid are removed by proper treatment with dichlorhydrin. Upon washing the separated organic solvents with successive small portions of water or with appropriate alkaline or acid solutions a separation of the constituent colours can in many cases be accomplished. The substance of Mathewson's method is as follows:

Separation of Colours by Means of Immiscible Solvents.—If the substance under examination is a solid insoluble in water, reduce it to a fine state of division and secure a solution of the colouring matter by macerating in

[1] *Bulletin* 147, *Bureau of Chemistry, U. S. Department of Agriculture.*
[2] *Bulletin* 162, *Bureau of Chemistry, U. S. Department of Agriculture.*

dilute (50–70%) alcohol made slightly alkaline with ammonia, or by a suitable method indicated on pages 649 to 663, Vol. V, under the heading of the respective food products. Dissolve the soluble substances in water. When working with solid samples advantage should be taken of any possible mechanical separation of portions coloured with different dyes. After a solution of the colour has been obtained, remove alcohol from the liquid by evaporating on a steam-bath, avoiding the formation of a dry residue by adding water.

A. *Separation of Basic Colours.*—Test a small portion of the dealcoholised liquid for basic colours by rendering alkaline with sodium hydroxide and shaking with ether. Separate the ether layer and shake it with dilute acetic acid. A coloured ether or acetic acid layer indicates the presence of a basic colour. In this case make the entire aqueous solution alkaline with sodium hydroxide and extract it with several successive portions of ether until the basic colour is all removed, as indicated by the fact that practically no colour is developed on shaking some of the last ether extract with dilute acetic acid. Shake the combined ether extracts with successive small portions of water, and finally with dilute acetic acid until colour is no longer extracted, keeping separate the aqueous layer obtained after each washing of the ether. A difference in the colour, fluorescence or other characteristic of these successive fractional washings indicates the presence of more than one basic dye. Combine the fractions containing the chief amount of each colour, make alkaline as before, shake out with ether, and subject the ether layer to fractional washing with water or dilute acetic acid as the case requires. By refractionating in this way some of each of the constituent basic dyes of a mixture may usually be obtained in a pure state and may be identified as indicated in Vol. V, p. 648. A tabular scheme showing the manner in which the dyes are removed from ether by the fractional washing is given on page 452.

B. *Separation of Acid Coal-tar Colours and Some of the Natural Colours.*— (a) Treat the original aqueous solution from which the basic dyes have been removed with half its volume of concentrated hydrochloric acid and shake with successive portions (about 25 c.c. each) of amyl alcohol until no more colour seems to be extracted. Not more than two or three extractions are usually required and the total volume of the amyl alcohol used need not exceed 50 to 75 c.c. Wash the combined amyl alcohol extracts with a little hydrochloric acid (1 part of strong acid and 2 parts of water) to remove sugar and similar impurities, the washings being rejected. Now shake the amyl alcohol with successive portions of water, the amount used each time being about half the volume of the amyl alcohol, until the washings are perfectly neutral, the water layer after each washing being run into a separate container. Eight or ten fractions will usually be obtained. Dilute the amyl alcohol with 1 or 2 volumes of gasoline or petroleum ether and shake out once or twice with water and finally with a very dilute sodium hydroxide

solution, the aqueous layers in this case also being kept each in a separate container. As in the case of the basic colours above described the presence of different dyes is indicated by a difference in colour, etc., of the successive fractional washings. Account must be taken at this point of the fact that some colours like Naphthol Yellow S. are more or less completely decolourised by mineral acid and also that many others are so modified in colour by different degrees of acidity that treatment of a small portion of each fraction with sodium bicarbonate is necessary to determine whether or not a colour or a mixture of colours is in fact present.

If a sufficient separation of the acid colours has not been effected by this treatment, combine the fractions containing the chief amount of each colour, acidify, shake up with amyl alcohol, separate the latter and wash with water (or hydrochloric acid of suitable strength) in the manner previously described. With the colours coming out last, use ether or petroleum ether instead of amyl alcohol in this purification. Occasionally in washing the amyl alcohol solution the liquids do not separate readily. In this case the mixture may be poured into a beaker and warmed, or hot water may be used. It is better, however, to use a centrifuge in breaking the emulsion because with hot mixtures a higher degree of acidity is required in order to extract the dye. Care should be taken at all times to keep the volumes of solutions as small as possible. The tabular scheme on page 452 shows the manner in which the acid dyes are removed from amyl alcohol by fractional washing. Identification of the separated colours is effected as indicated in Vol. V, p. 648.

The *natural colouring matters* archil (unsulphonated), turmeric, and saffron are taken up by the amyl alcohol when shaken with this solvent as under B(a) and are not removed until, after dilution with gasoline, the mixture is shaken with dilute sodium hydroxide. Separate the alkaline solution, acidify slightly with hydrochloric acid, and shake out the colour with amyl alcohol. Evaporate the amyl alcohol extract to dryness on a steam bath and test the residue for identity of the colour as indicated on page 632, *et seq.*, of Vol. V. Cochineal and Persian berry are also taken up by the amyl alcohol under B(a) but are gradually removed by the washings with water and completely by the fraction obtained after dilution with gasoline. To obtain a comparatively concentrated solution of these two colours for purposes of identification unite the fractions containing them, acidify with hydrochloric acid and shake with amyl alcohol. Separate the latter, dilute with 2 volumes of gasoline and shake with a little water. The colour passes into the water layer and may be identified as indicated on page 632, *et seq.*, of Vol. V. Some of the natural colouring matters are rendered much paler by acids and may be overlooked in the fractional washings. The acid solutions of most natural colours are deepened in tint by adding stannous chloride, while most of the commonly used coal-tar colours are decolourised, and it is therefore advisable to test a few drops of the strongly coloured fractions with this reagent.

(b) The original mixture from which the basic colours have been removed with ether, and most of the acid colours with amyl alcohol which may appear perfectly colourless, may still contain Light Green S. F. Yellowish and some other dyes.   Make the mixture slightly alkaline with sodium carbonate or ammonia and then acidify slightly with acetic acid.   Shake once or twice with amyl alcohol to remove any Guinea Green, Methylene Blue, etc., that may be present.   Then extract with one or two small portions of dichlorhydrin which removes Light Green S. F. Yellowish and similar strongly sulphonated triphenylmethane greens.   Separate the dichlorhydrin, dilute with double its volume of benzene and wash out the colour with water.

Outline of the Manner in which the Coal-tar Colours Behave when Shaken with Various Solvents under Conditions Given in the Above Scheme.[1]—In the colour separations as described, any given dye will in general, appear in several washings and the table given below is therefore only designed to show where the maximum amount will come out.   The numbers refer to the dyes as listed in Green's edition of Schultz and Julius, *Systematic Survey of Organic Colouring Matters*, 1904, and are identified by name on page 641 of Vol. V, this edition, with the six exceptions here given.

2–Victoria Yellow.	7–Aniline Yellow.	491–Wool Green S (B).
3–Martius' Yellow.	62–Palatine Red (B).	602–Nigrosin Soluble (A).

A. Basic dyes.   Extracted by ether from strongly alkaline solutions. (Extracted only in small amount, perhaps with decomposition: 650.)

   (1) Readily removed from ether by washing with water: 448, 584.
   (2) More or less slowly removed by water, quickly by acetic acid: 425, 427, 428, 451, 452, 504, 655.
   (3) Not removed by water, fairly readily removed by acetic acid: 17, 18, 197, 201.
   (4) Not removed by acetic acid, fairly readily removed by hydrochloric acid (oil-soluble colours): 7, 16.
   (5) Not removed by hydrochloric acid (oil-soluble colours): 11, 49, 60.

B. Acid dyes.   Not extracted by ether from alkaline solution.
   a. *Extracted by amyl alcohol from strongly acid solutions.*
   (1) Removed in first washings of amyl alcohol extract, acidity high: 8, 9, 89, 108, 692.
   (2) Removed at lower acidity, but usually above fourth normal: 94, 106, 107, 602.
   (3) Removed at rather low acidity: 14, 53, 188, 480.
   (4) Removed at very low acidity, but before washings are neutral. Like preceding acid colours, not extracted by amyl alcohol from 5% sodium chloride solution.
      (a) Removed from strongly acid solution by amyl acetate: 4.
      (b) Not readily removed by amyl acetate: 55, 56, 62, 64, 65, 84, 103, 105, 139.

[1] W. E. Mathewson, *loc. cit.*

(5) Removed by water from the practically neutral solvent, most readily after addition of petroleum ether.

    (a) Not completely extracted by amyl alcohol from 5% sodium chloride solution: 146, 169, 667.

    (b) Almost completely extracted:

        (1) Extracted by 5% sodium carbonate solution from amyl alcohol: 85.

        (2) Not readily extracted: 13, 86, 95, 97, 101, 137, 329 (464, 468, 433).

(6) Removed by diluted sodium hydroxide solution from the amyl alcohol-petroleum ether mixture. Readily extracted by ether from acid solutions: 2, 3, 269, 510, 512, 516, 517, 518, 520, 523.

b. *Not extracted from the strongly acid solution by amyl alcohol.*

    (1) (Decomposed: 398.)

    (2) (Dye separates as a precipitate but is extracted by dichlorhydrin: 240, 602.)

    (3) After adding ammonia until nearly neutral:

        (a) Readily extracted by amyl alcohol: 433 464, 468 (650).

        (b) Not readily extracted by amyl alcohol:

            (1) Extracted by dichlorhydrin: 434, 435, 440, 491.

            (2) Not readily extracted by dichlorhydrin: 462.

Separation of the Seven Colours Permitted in the United States.—In the routine examination for colours as described above all the permitted dyes excepting Light Green S. F. Yellowish will be extracted by the amyl alcohol under B (a). Upon washing the amyl alcohol with water the different colours will appear in the washings in the following order: Indigo Carmine, Amaranth, Ponceau 3R, Naphthol Yellow S, Orange I, and Erythrosin. The separation of the Indigo Carmine and Amaranth by the fractional washing is quite sharp, that of Ponceau 3R and Naphthol Yellow S not so much so, while the great bulk of Orange I and Erythrosin remains in the amyl alcohol until after dilution with gasoline. The combined fractions containing the bulk of the Naphthol Yellow S and Ponceau 3R are treated with about one-eighth their volume of concentrated hydrochloric acid and shaken with two or three successive portions of amyl acetate which removes the Naphthol Yellow S leaving the Ponceau 3R in the aqueous layer. Strongly acidify the separated aqueous layer and shake the colour into a little amyl alcohol. Wash the amyl alcohol with a little $N/4$ hydrochloric acid, dilute with 2 volumes of gasoline and shake out the colour with a little water. Wash the amyl acetate containing the Naphthol Yellow S once with dilute hydrochloric acid (1 volume of concentrated hydrochloric acid and 9 volumes of water) and then remove the colour by washing with water. Orange I is removed from the original amyl alcohol extract by diluting with gasoline and washing with water. Any trace of Erythrosin can be removed from these washings by acidifying with acetic acid and shaking with ether. The Erythrosin re-

maining in the amyl alcohol-gasoline is removed by shaking with dilute sodium hydroxide solution. It may be purified by acidifying the alkaline solution and shaking with ether. Upon shaking the ether extract with dilute ammonium hydroxide the colour passes into the aqueous layer. Light Green S. F. Yellowish remains in the original aqueous layer after shaking the strongly acid solution with amyl alcohol as in B(a). It is removed after neutralising most of the free acid, by shaking with dichlorhydrin as in B(b3). After diluting with benzene the colour is removed by washing with water.

The three colours Ponceau 3R, Naphthol Yellow S, and Orange I can be most readily separated by treating their aqueous solution with one-fourth its volume of sodium chloride solution (250 grm. per 1,000 c.c.) and shaking with one or two separate portions of amyl alcohol which removes the Orange I. Wash the amyl alcohol with 5% salt solution to remove any Yellow or Ponceau, and then two or three times with 5% sodium carbonate solution. The Orange I passes into the sodium carbonate solution leaving any Orange II, Crocein Orange, etc., that may be present in the amyl alcohol from which they may be removed by diluting with gasoline and shaking with water. Treat the combined salt solutions from which the Orange has been removed with one-tenth to one-fifth their volume of concentrated hydrochloric acid and shake with two or three successive portions of amyl acetate which takes up the Yellow. Remove the colour from the amyl acetate by washing with water. Shake the salt solution containing the Ponceau with amyl alcohol, separate the solvent, wash once with a little water, dilute with gasoline, and shake out the colour with a little water.

The solution of Ponceau 3R as obtained above should give a deep purplish-red precipitate on treatment with a few drops of barium acetate solution all of the colour being precipitated. Ponceau 2R also gives a precipitate under these conditions but its colour is carmine red. Amaranth can be distinguished from the Ponceaus and Fast Reds by its behaviour in acid solution when shaken with amyl alcohol. A dilute solution of the colour in $N/4$ hydrochloric acid when shaken with an equal volume of amyl alcohol will retain most of the Amaranth in the aqueous layer, Ponceau 3R on the other hand passing into the amyl alcohol. Indigo Carmine is not readily taken up from slightly acid solutions by dichlorhydrin which distinguishes it from common blue and green triphenylmethane and azin dyes.

Read's Test for Detecting Colour in Tea.[1]—This test has the advantages of simplicity and ease of manipulation as well as being capable of detecting smaller amounts of pigment than the methods given in Vol. V. Place about 25–50 grm. of tea in a sieve having 40 to 60 meshes to the inch and shake over a piece of white paper. If the tea is tightly rolled it should be slightly crushed, either before putting into the sieve or by rubbing it against the latter. Drag a spatula or the blade of a case knife flat side downward over the paper crushing the dust between the blade and the paper. A

[1] E. A. Read, Vol. 18, *Orig. Communications, 8th Intern. Congress App. Chem.*, p. 301.

little pressure applied by the finger to the end of the blade will be necessary to secure the proper crushing or streaking effect. Any particles of pigment in the dust will be revealed by a coloured streak on the white paper. If black unglazed paper be used white facing pigments can be detected in the same way by the appearance of white streaks. A lens with a magnification of 8 to 12 diameters is useful in detecting the smaller streaks. Observation of the streaks must be made in bright daylight, sunlight being desirable.

The *character of the pigments* present may be determined as follows: A black glossy streak indicates carbon. A blue streak may be due to prussian blue, ultramarine or indigo. Treat the colour either directly on the paper or after transferring to a microscopic slide, with a drop of 40% sodium hydroxide solution. Prussian blue will become yellowish-brown; indigo or ultramarine will remain unchanged in colour. Treat another portion of the streak with 10% hydrochloric acid. The blue colour of ultramarine is discharged; indigo remains unchanged. A yellow streak may indicate turmeric. This becomes bright red upon treatment with a mixture composed of concentrated hydrochloric acid saturated with boric acid. Concentrated sulphuric acid also turns turmeric bright red.

ERRATA IN VOL. V.

Page 636, line 17 from bottom for " Tumeric" read " Turmeric."
Page 648, line 12 from bottom, for "435 to 438" read "460 and 489."
Page 668, line 3 from bottom, in table, for "naphthaline" read "naphthalene."

PRINTING INKS.

By JOHN B. TUTTLE.

Historical.—The first printing inks were made by incorporating lampblack with burnt linseed oil. Later to meet the requirements of new conditions of presses, paper, etc., other oils, such as rosin oil, mineral oil, the semi-drying oils, China wood oil, etc., were introduced, whilst gas black, bone black or ivory black, magnetic pigment, etc., replaced the original lampblack. Colour printing required a great variety of pigments, divided roughly into two classes, the inorganic pigments, and the coal-tar dyes and lakes.

Composition.—Printing inks may be divided into two parts: first, a vehicle, or varnish; and second, the pigments.

The composition of the vehicle is determined by the nature of the work for which the ink is intended. It may contain linseed oil, mineral oil, rosin oil, the semi-drying oils, China wood oil, certain bituminous substances, hard gums or resins such as kauri and copal, rosin (colophony), various potassium, sodium, calcium and aluminium soaps, and one or more of a large variety of driers.

The linseed oil used in printing inks is specially prepared for the purpose by one of two methods. The older method, still in use in preparing oil for engraving inks, consists in heating the oil in an open kettle, setting fire to the fumes, and allowing the oil to burn until the desired consistency, or viscosity, is attained. The second method is to heat the oil in an open kettle at about 300° without allowing the vapours to inflame. · In both processes a number of grades are prepared by varying the length of time of heating.

The rosin is added to the vehicle either as such, or in the form of the calcium soap. The driers are usually lead, manganese or cobalt salts of organic acids. The other constituents of the vehicle are used without special treatment.

The pigments used will depend not only upon the colour desired, but also upon the class of work. For example, in printing with one colour an opaque pigment is necessary so that the colour of the paper will not show through the ink, whilst a translucent pigment is required in the three- and four-colour processes, where the secondary colours are obtained by superimposing the primary ones.

The principal black pigments are lampblack, gas black (or carbon black), bone black, and artificial magnetic oxide of iron. The inorganic

coloured pigments include Prussian blue,[1] ultramarine, chrome yellow (including all the various hues from pale yellow to scarlet), chrome green, vermilion, etc. The organic pigments include the various coal-tar lakes and dyes.

Manufacture of Ink.—The mechanical part of the manufacture of printing inks is quite simple. The vehicle is prepared according to the desired formula and then the pigments are added. There is usually a preliminary mixing in a mill containing broad revolving knives or paddles, after which the inks are ground in a grinding mill. This latter consists of three rolls which revolve at different speeds, the rear one slowest, the front roll fastest. The ink is fed between the rear and middle rolls, is carried around by the middle to the front roll, where it is automatically scraped off. The differential speed gives the grinding effect and reduces the pigment to the finest division possible. Many of the better grades of ink are ground a number of times before they are considered satisfactory.

Analysis.—There has been very little work published on the analysis of printing inks. The methods which are given below are taken largely from the article on this subject by J. B. Tuttle and W. H. Smith.[2] The general procedure consists in separating, by means of suitable solvents the inks into two parts, oils and pigments, and testing the separate parts for the constituents which are likely to be present.

For inks in which the vehicle is largely, if not entirely, mineral or rosin oils, petroleum ether will be found a very satisfactory solvent, but for general use where the composition is unknown, a mixture of 3 parts ethyl ether and 1 part benzene is to be preferred. The separation is best performed by centrifuging; the settling process consumes too much time to be practicable.

Separation of Oil from Pigment.—About 50 grm. of ink (avoiding the hard film which frequently forms on the surface) are placed in a weighed glass tumbler of about 300 c.c. capacity, a small amount of solvent added, and the whole stirred thoroughly until a homogeneous mixture is obtained. The glass is then filled with the solvent to within about $\frac{1}{2}$ in. from the top, and the whole again stirred. It is next placed in the metal cup of the centrifuging machine and the space between the glass and metal cups filled with water in order to equalize the pressure of the liquid inside the glass during the centrifuging. Placing a rubber disc at the bottom of the metal cup has been found to materially lessen the danger of breakage during the operation. The metal cup and contents are then exactly counterbalanced most conveniently by either a second sample of the same ink or another sample of ink, and then both are placed in the machine. For web-press and flat-bed inks, 2,000 revolutions per minute for 10 minutes will suffice for a

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[1] The term Prussian blue is here used to denote any of the iron cyanide blues, such as Milori, bronze, Chinese blues, etc., which may be used in printing inks. These names are applied more or less indiscriminately; the simplest procedure, therefore, is to use the better known term "Prussian blue" to cover all of them. Even if there were a standard nomenclature, it is doubtful if they could be identified after incorporation in a printing ink.

[2] *Technologic Paper No. 39, of the U. S. Bureau of Standards*, "The Analysis of Printing Inks."

complete separation. Where gas black has been used, it has frequently been necessary to run the machine at 2,600 to 2,800 revolutions per minute for 20 or 30 minutes before a satisfactory separation is secured. The clear liquid is decanted through a pleated filter into a glass bottle, a further quantity of solvent added, and the process repeated. Usually three treatments suffice to give practically complete separation of oil and pigment. The glass and contents are dried at about 90° and on cooling, reweighed. The increase in weight is the pigment, which is calculated as a percentage. The amount of pigment on the filter paper should be negligible if the centrifuging has been efficient.

This method will not always yield results of great accuracy. The errors, which vary in magnitude with different inks, are as follows:

Some of the dyes are soluble to some extent in the solvents, tending to give low results for pigment.

Hard gums may not be completely soluble, and thus part will remain with the pigment.

The hard scum (linoxyn) which forms on the surface of the ink after it has been exposed awhile, is difficultly soluble and remains with the pigment. This should be excluded in sampling, for if it is not done, a considerable error may be introduced.

Carbon black contains some particles so fine that it is impossible to cause them to settle, even in the centrifuge.

The net error of this separation is therefore the algebraic sum of these various errors.

Analysis of the Oil.—The oil fraction may contain any of the oils, etc., mentioned in the paragraph on the composition of inks. Bituminous substances are judged largely by colour, being a mixture of a number of different substances of varying chemical nature; the estimation of the total amount present is a matter of too much difficulty to justify the time required.

Oil Constants.—Estimating the oil constants, such as iodine number, saponification number, acid number, etc., does not give very reliable data regarding composition. If there were but two components, the proportion of each might be estimated at least approximately in this way, but with three, and sometimes more substances present, such methods are useless, even if the constants of the individual substances are well known.[1]

We are therefore forced to rely upon qualitative tests, supplemented by quantitative estimations of some of the more important constituents. The oil fraction of an ink is independent of the colour; therefore the separation given below is applicable to inks of all colours.

[1] The various linseed varnishes used in printing inks have widely varying constants and the estimation of these is a very difficult problem. Smith and Tuttle have shown (*Technologic Paper No. 37 of the U. S. Bureau of Standards*, 'The Iodine Number of Linseed and Petroleum Oils"), that slight changes in the method used in estimating the iodine number of the so-called "burnt" linseed varnishes will give widely varying results. It would seem an almost hopeless task to attempt to determine just which varnish has been used, and the difficulties become even greater when, as is frequently the case, *two or more of them are used in the same ink.*

It will be found convenient to regard the oil fraction as consisting of hard gums, rosin, unsaponifiable matter (rosin and mineral oils) and linseed oil.

Hard Gums.—The hard gums are difficult to estimate, the only method which has given any measure of satisfaction being that of McIlhiny.[1] This method depends upon the insolubility of hard gums in water and petroleum ether. The method is much better adapted for the analysis of paints than printing-ink varnishes, but it can be used for the latter to obtain some idea of the amount present.

Unsaponifiable Matter.—Sufficient of the solution from the separation of the oil and pigment to leave a residue of about 5 grm. is evaporated in a weighed beaker; 50 c.c. of normal alcoholic potassium hydroxide is added, the beaker covered with a watch glass, and heated on a steam-bath for several hours, being stirred frequently to assist saponification. When the latter is complete, the watch glass is removed and the alcohol distilled off. The residue is transferred to a separating funnel with successive portions of water, in all about 100 c.c. being used, and extracted with petroleum ether until no further oil can be removed. Four extractions are usually sufficient. The petroleum ether fractions are united in another funnel, washed with water until the wash-water is no longer alkaline, filtered into a weighed beaker, the petroleum ether distilled off and the residue dried at 95°, cooled and weighed. If this unsaponifiable matter is over 2%, it indicates the presence of something else than linseed oil and hard gums. The wash-water from the first two washings should be united with the water layer in the first separating funnel.

Rosin.—This unsaponifiable matter is tested for the presence of rosin oil. The most satisfactory method of testing qualitatively for this material is the Liebermann-Storch test, which consists in heating a small portion of the oil with 10 c.c. of acetic anhydride, allowing it to cool, and adding a drop of sulphuric acid of sp. gr. 1.63. A violet colouration indicates rosin oil. It is always best to carry out a control test at the same time with some pure rosin or rosin oil. The test is identical for the two materials.

If the test for rosin oil is positive, the alkaline aqueous solution which has been extracted with petroleum ether is made acid with hydrochloric acid (there is usually sufficient dye present from the ink to act as indicator), and the fatty acids which are thus liberated are extracted with successive portions of ethyl ether. These extracts are united, washed free from acid and salts and evaporated in a small beaker.

A quantitative determination of the rosin can be made either by the Twitchell method, which depends upon the separation of the esters of the organic acids, or by Gladding's method,[2] which depends upon the separation of the silver salts of these acids.

A very satisfactory method is Parry's modification of Gladding's method.[3]

[1] P. C. McIlhiny, *Chem. Eng.*, 1908, **8**, 70; *Chem. Abs.*, 1908, **2**, 2630.
[2] *Amer. Chem. Jour.*, 1881, **3**, 416.
[3] Vol. IV, p. 73.

The fatty acids are dissolved in 20 c.c. of 95% alcohol, a drop of phenol-phthaleïn added and then a strong solution of sodium hydroxide (1 part alkali to 2 water) until the reaction is just alkaline. The solution is heated for a few minutes, allowed to cool, and then transferred to a 100 c.c. stoppered graduated cylinder. The latter is filled to the 100 c.c. mark with ethyl ether, 2 grm. of powdered silver nitrate crystals are added, and the mixture shaken vigorously for 15 minutes, in order to convert the acids into their silver salts. When the insoluble salts have settled, 50 c.c. of the clear solution (containing the silver salts of rosin) are pipetted off into a second 100 c.c. cylinder, and shaken with 20 c.c. of dilute hydrochloric acid (1 acid to 2 water). The ethereal layer is drawn off, and the aqueous layer is shaken twice with ether. The ether extracts are united, washed with water, and the ether distilled off in a weighed beaker. The residue (rosin) is dried at 110° to 115°, cooled, and weighed. The results are calculated on the basis of the original weight of the oil.

The difference between 100 and the sum of the unsaponifiable matter (if over 2%) and the rosin, may be considered linseed oil.

Analysis of the Pigment.

Black Inks.—A mixture of oil and black pigments will not give a pure dense black, owing to the various undertones of the pigments. Moreover, the public is accustomed, in printing, to accept as black what is really a blue-black. Practically all of the pigments from black inks will be found to contain more or less blue, either in the form of Prussian blue, or blue dyes and lakes.

Ashing.—The first step in the analysis of the pigment of a black ink is to ignite a weighed quantity in a porcelain crucible (platinum cannot be used on account of the lead which is usually present). The ignition should be performed at the lowest possible temperature required to obtain complete combustion. This precaution is general and applies to all inks. The loss on ignition represents lampblack, the carbon of the bone-black (should there be any present), aniline dyes, and undissolved oils and gums. Prussian blue is decomposed by heat, part of it being volatilized, the iron remaining behind as ferric oxide. The residue from the ignition contains any added mineral matter of the pigment, lead or manganese from the driers, ferric oxide from the Prussian blue, or ferric oxide added as such (the so-called magnetic pigment), calcium phosphate if bone-black is present, and alkali or calcium carbonates from the soaps present. All ignitions of pigment must be performed under a hood having a strong draught.

Prussian blue should be tested for qualitatively in the dry pigment. For this purpose, 1 grm. of pigment is moistened with 2 or 3 c.c. of normal alcoholic potassium hydroxide, heated on the steam-bath until the alcohol is removed, 5 c.c. of water added, and the insoluble matter filtered off. The

filtrate is made acid with hydrochloric acid and filtered again if necessary. When ferric chloride is added a blue precipitate will be obtained if Prussian blue is present. Sometimes sufficient blue dye goes through the filtrate to obscure the indication. In this case the solution is again made alkaline and filtered. After filtration it is made acid with hydrochloric acid as before and then copper sulphate is added. The precipitate is filtered and washed thoroughly, and consists of reddish-brown copper ferrocyanide. It is advisable, in case of doubt, to add a small amount of Prussian blue to the pigment, and make a control test. The ash is analysed quantitatively for insoluble matter, lead, iron, manganese and calcium.

Insoluble Matter.—0.250 grm. of the ash is heated to dull redness in a porcelain crucible for a few minutes, cooled in a desiccator, and weighed. It is transferred to a 250 c.c. beaker, concentrated hydrochloric acid being used to dissolve any material that may stick to the crucible. About 25 c.c. of concentrated hydrochloric acid are added, the beaker covered with a watch glass, and after heating until as much as will go in solution is dissolved, the cover is removed, and the solution evaporated to dryness. The residue is moistened with a few drops of concentrated hydrochloric acid, 50 to 75 c.c. of boiling water added, and the solution is filtered, washing thoroughly with hot water. The filter paper and residue are ignited and weighed, and the product called "*insoluble matter.*"

Lead.—50 c.c. of 10% sulphuric acid are added to the filtrate from the previous estimation and evaporated down until the solution fumes strongly. This is cooled, diluted carefully with about 100–150 c.c. of water and heated on the steam-bath until any basic ferric sulphate which sometimes separates is redissolved. The precipitate, containing the lead sulphate, is now filtered off. A small amount of lead sulphate will, in all probability, remain in solution, but inasmuch as the ash is seldom more than a few per cent. of the entire ink, and of this only a small amount is lead, the amount thus lost is negligible. The precipitated lead sulphate is dissolved in ammonium citrate or acetate, filtered from any insoluble matter, the filtrate made strongly acid with sulphuric acid, and the precipitated lead sulphate filtered off on a Gooch crucible, ignited and weighed. A platinum Gooch crucible with a platinum felt will be found extremely satisfactory. The insoluble matter from the ammonium acetate solution should be examined for calcium and barium.

Another method for the estimation of the lead is nearly to neutralise the acid present with sodium carbonate, saturate the solution with hydrogen sulphide, filter off the precipitated lead sulphide, dissolve it in fairly strong nitric acid and determine the lead as sulphate by adding sulphuric acid as above described. In this case solution in ammonium acetate is omitted. The former method is advantageous when qualitative tests show that there is very little manganese present, and it is desired to estimate only the iron. After the lead sulphate has been removed the solution obtained is in perfect condition for this estimation.

Iron.—The iron in the filtrate from the lead sulphate is reduced to the ferrous condition by passing the solution through a Jones reductor, and the ferrous sulphate titrated with a standard solution of potassium permanganate.

Iron is separated from manganese and other metals which may be present by precipitating with ammonium hydroxide, the precipitate being filtered off, redissolved in hydrochloric acid, reprecipitated with ammonium hydroxide and again filtered. It is now dissolved in hydrochloric acid, sulphuric acid added, and the solution evaporated until all the hydrochloric acid is removed; it is diluted and the iron estimated as before, with the Jones reductor. This method is rapid and accurate. Before adding ammonium hydroxide, if hydrogen sulphide has been used, the solution should be boiled until all the hydrogen sulphide is removed, and nitric acid added to oxidise the iron to the ferric condition.

Manganese.—Hydrogen sulphide is now passed into the ammoniacal solution from the iron precipitation. This is allowed to stand over night, and the precipitate, if there is any, is examined for manganese. Usually there is only a trace of manganese, insufficient to warrant a quantitative estimation. Should there be much manganese, the sulphide can be filtered off, and the quantitative estimation made by conversion into the pyrophosphate.

Calcium.—If it is desired to estimate the calcium, this can be done after filtering from the manganese sulphide. (If phosphates are present, as for instance, if bone-black is present, a basic acetate separation is required.) In either case, the lead should be separated by hydrogen sulphide. The filtrate from the manganese sulphide is heated on the steam-bath until the hydrogen sulphide is removed, ammonium hydroxide and ammonium oxalate are added, and the precipitated calcium oxalate is estimated either as calcium oxide or sulphate.

Nature of the Pigment.—The percentage of ash will be of great assistance in determining the nature of the pigment. Black oxide of iron is only slightly changed on heating, being completely oxidised to ferric oxide. Bone-black is composed largely of calcium phosphate, yielding the greater part of its weight as ash. The presence of any large amount of phosphoric acid will be sufficient evidence that bone-black has been used.

In the absence of black oxide of iron, we may assume that all of the iron in the filtrate is due to the Prussian blue. The percentage of Fe_2O_3 in the ash, multiplied by the percentage of ash in the pigment, multiplied by the factor 1.53 will give roughly the amount of Prussian blue present. The factor 1.53 is obtained from the ratio $Fe_7(CN)_{18}$ to Fe_2O_3. It is purely theoretical and is probably low, but is sufficiently accurate for most purposes.

When the presence of oxide of iron is suspected, 1 grm. of pigment is wrapped in filter paper, and the dye extracted with alcohol, using for this purpose an extractor of the Wiley type, in which the material is extracted by the solvent at its boiling point. When all the dye has been extracted, the paper and contents are dried, and the nitrogen is estimated in the residue by

the Kjeldahl method. From the nitrogen thus obtained, the Prussian blue is calculated, using the factor 3.41. The Fe_2O_3 present in this amount of Prussian blue is deducted from the total Fe_2O_3 found in the ash. The remainder will be the percentage of iron from the magnetic oxide. The formula of the latter is theoretically Fe_3O_4, and the proper calculation should be made. This method for the estimation of Prussian blue depends upon the fact that the aniline dye is the only other material which may contain nitrogen. Instead of calculating the Prussian blue from the amount of iron present, it is estimated from the nitrogen remaining after the removal of the aniline dye. In this way both Prussian blue and magnetic oxide of iron may be estimated with reasonable accuracy.

Dyes.—Practically all the dyes which are used in black printing inks are soluble in alcohol, so that an approximate estimation can be made by extracting the pigment with this solvent. This method is the same as described in the preceding paragraph, the alcoholic solution being evaporated off in a weighed beaker, dried at 90°, cooled and weighed.

Blue Inks.

A weighed quantity of pigment is ignited as under black pigments The ash is analysed by the same process as before, determining only lead, manganese, and iron if the qualitative tests show that Prussian blue is present. The lead and manganese are reported as metallic driers, the iron is calculated as Prussian blue, and the remainder reported as mineral filler. The composition of the filler, as a rule, is of no consequence.

Ultramarine.—The presence of ultramarine will be shown by the blue colour of the ash. Hydrogen sulphide is evolved from the latter on the addition of hydrochloric acid. There is, unfortunately, no method for its quantitative estimation. In this case, the ash is reported after deducting the lead and manganese.

Soluble aniline dyes are estimated by extraction with alcohol as under black pigments.

Red Inks.

Vermilion.—The most brilliant red mineral pigment is unquestionably vermilion (mercuric sulphide). Its price prohibits its use except in inks used for special purposes. It is very readily detected qualitatively by covering a small quantity of pigment with 4 or 5 c.c. of aqua regia, and heating gently. The solution is diluted with 5 volumes of water, filtered, and stannous chloride added to the filtrate. A grayish precipitate of mercury will be formed if vermilion is present. A very small amount can be readily detected by this test.

The quantitative estimation of vermilion, however, is much more difficult. One method is to dissolve the mercuric sulphide in aqua regia, and

after nearly neutralising the diluted solution, to precipitate the mercuric sulphide with hydrogen sulphide and weigh the precipitate on a Gooch crucible, observing all the precautions to eliminate sulphur which separates during the precipitation.

The following process has also been found of value: 1 grm. of the pigment is treated with a slight excess of ammonium sulphide. Sodium hydroxide is then added, whilst stirring. The beaker is placed upon the steam-bath, more alkali is added if necessary, until all the mercuric sulphide passes into solution. An undue excess of alkali should be avoided.· The solution is allowed to cool, filtered, and the residue washed thoroughly. To the filtrate, sufficient ammonium nitrate to reprecipitate the mercuric sulphide is added, and it is then boiled to expel ammonia. The precipitate is allowed to settle, which takes but a short time, and the supernatant liquid decanted through a weighed Gooch crucible. The residual mercuric sulphide is boiled with a little sodium sulphite solution to remove free sulphur, and is then transferred to the crucible, where it is washed with hot water until it no longer reacts with silver nitrate solution. It is dried at 110° and weighed.

The distillation method, in which the mercury is absorbed by gold, and the various electrolytic methods will appeal to those who have had experience with them.

Metallic Driers.—The pigment is ignited and the ash analysed for lead and manganese. The remainder of the ash is reported as mineral filler.

Green Inks.

The colouring matter may be chrome green, green lake or dye. Some of the darker shades are obtained by the addition of lampblack.

Ash.—The ash of the pigment is estimated as usual. Part of this ash is tested qualitatively for chromium. If present, the ash should be tested for the following substances: lead chromate, lead sulphate, lead oxide, barium sulphate, calcium sulphate, ferric oxide, and oxides of manganese.

Sulphur.—To estimate sulphur, 0.250 grm. of the ash and 5 grm. of a mixture of equal parts of potassium nitrate and sodium carbonate are fused in a porcelain crucible over a sulphur-free flame. The cooled mass is extracted with hot water and filtered. The filtrate is acidified with hydrochloric acid, heated to boiling, and 10 c.c. of 10% barium chloride solution are added. After standing over night, the precipitated barium sulphate is filtered off, ignited, and weighed as usual. The solution should be sufficiently acid to prevent any significant contamination of the barium sulphate with barium chromate.

Barium.—To estimate barium, the insoluble matter from the determination of sulphur, is dissolved in hydrochloric acid, the solution made nearly neutral with sodium carbonate, and hydrogen sulphide is passed into the solution until all the lead is precipitated. The lead sulphide is filtered

off, the filtrate heated to boiling, and 10 c.c. of 10% sulphuric acid are added. The barium sulphate is treated as directed under the determination of sulphur.

Chromium.—A fresh portion of ash is mixed with sodium peroxide, and fused in a nickel crucible. The cooled melt is dissolved in hot water and filtered. Carbon dioxide is passed into the filtrate, and the latter heated again on the steam-bath in order to precipitate any lead which may have been held up by the sodium hydroxide. Any insoluble matter which may separate is filtered off. The filtrate is made strongly acid with hydrochloric acid, potassium iodide added, and the liberated iodine titrated with a standard sodium thiosulphate solution. From the amount of thiosulphate used, the amount of chromium oxide present is calculated.

The two precipitates from the estimation of chromium are combined, and used for the estimation of lead, iron, manganese and calcium. They are dissolved off the filter paper with hydrochloric acid, the solution is nearly neutralised with sodium carbonate, and hydrogen sulphide passed into the solution. The precipitated lead sulphide is filtered off, dissolved in nitric acid, and estimated as sulphate as directed under black pigments. The filtrate from the lead sulphide is treated for iron, manganese, and calcium, as directed under black pigments. Usually only the iron is of sufficient importance to warrant a quantitative estimation.

It is difficult to give precise directions for calculating the results from the preceding estimations. To a large extent the analyst must use his experience in deciding the various questions as they arise. It is probably safe to assume that all of the chromium was originally present as lead chromate, and it should be so calculated. The iron oxide should be calculated as Prussian blue provided there is a positive qualitative test. Any barium present should be calculated as sulphate; if there is any question as to its being originally present as carbonate, the ash of the pigment is treated with very dilute hydrochloric acid, the solution filtered, and the filtrate tested for barium. Barytes is difficultly soluble in cold dilute hydrochloric acid. In the absence of barytes, the sulphur present is calculated as lead sulphate. The excess of lead over that required for the lead chromate and sulphate, may be considered as drier.

China clay may be present, either as an added part of the chrome green, or as the base of a green lake. Aluminium hydroxide is also used as a base for coal-tar lakes. In such cases, the unestimated portion of the ash should be reported as lake base or mineral filler.

Green dyes are estimated by extraction as usual. In the absence of chrome green, the pigment is ashed, and the ash analysed for lead and manganese only, the remainder being reported as mineral fillers.

If lampblack has been used to produce a dark shade of green, it can be tested for qualitatively by taking a small portion of the pigment, treating it with strong alkali, and filtering through a Gooch crucible, washing first with

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hot water, and finally with moderately strong hydrochloric acid. Lampblack will show a black residue, which will disappear on ignition. No quantitative estimation has been developed for this material, and it is generally classed with the volatile constituents, which are then reported as aniline dye, lampblack, undissolved oil, etc.

Inks of Other Colours.

The above classes represent the inks most used for ordinary work. If it is desired to test other colours, the general procedure would be simply to make qualitative tests for the pigments. The metallic driers present can be determined in the ash if so desired. References to the various text-books on this subject may be of assistance in suggesting what materials may be present.

Permanence to Light.

With coloured inks, the question of importance is frequently not so much what dye or lake has been used, and how much, but how permanent it is. Exposure to light is the easiest method for determining this. This test is performed by making some streaks on white paper with the ink in question. These should be about $\frac{1}{2}$ in. wide, and about 10 in. long. The film of ink should be as thin as it is possible to make it, and should correspond as nearly as possible to the thickness of the film of ink used in printing. The sheet is allowed to remain in a dark place for 24 hours to dry thoroughly, and is then divided into three parts. The middle section is exposed to direct sunlight until the colour changes, or until it is apparent that no change will take place, 50 to 75 hours being about the right length of time. The two outside sections are kept in the dark, for the purpose of comparison. After the exposure is completed, the strips are joined together in their original position, when it is possible to detect very slight changes in colour. A number of inks can be tested on the same sheet if so desired.

Another method for determining the relative permanency of different samples of the same colour has been suggested.[1]

Flat tints of each ink are printed as strongly as if they were to be part of a colour job. These tints should be about 5 in. by 7 in. in size. They should then be cut out to this size. A photometer scale is then made of five layers of fine tissue paper, each layer 1 in. narrower than the preceding. This will give five different thicknesses of tissue, each thickness representing a band 1 in. by 7 in. Across these, and about 1 in. apart, should be glued three strips of opaque black paper, 1 in. wide and 5 in. long, starting 1 in. from and parallel to one narrow edge of the tissue paper. The photometer thus made, and a printed sheet of the ink to be tested, are then put into a

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[1] Private communication from Mr. H. R. Gaylord and Mr. Averill, of the State Institute for the study of Malignant Disease, at Buffalo, N. Y., through Mr. E. S. Moores of the U. S. Government Printing Office at Washington, D. C.

photographic printing frame which has a plain glass in the front of it. The whole is then exposed to the sunlight with an ordinary photographic printing-out photometer until the total exposure has reached a certain value on the photometer scale (usually the last number). When the printed sheet is taken from the frame, it will be found to be divided into three unfaded areas, corresponding with the three opaque black strips, and four faded areas, each divided into five 1-in. squares which have each received different amounts of light. By placing sheets of different printing inks of the same colour in the frame, and exposing to sunlight to the same photometer number, the relative permanency of the different inks can be seen at a glance. If desired, three inks may be tested at one time by cutting the flat tints into strips 2 in. wide and 5 in. long, and placing them in the frame so that each has an exposed and an unexposed area.

Dyes and Lakes.

So far, little success has been met with in attempting to estimate the various coal-tar lakes used in printing inks. The soluble dyes may be removed by extraction, and the amount present estimated in this way. Some of these may be identified by the number and location of the absorption bands, using for this purpose the tables given by J. Formanèk in his book on "*Spektranalytischer Nachweis Künstlicher Organischen Farbstoffe.*"

Special Tests.

The foregoing tests cover practically all the important components of the common inks. A few other tests might be made in case of trouble that cannot otherwise be located.

Volatile constituents in the ink can be determined by placing a weighed quantity in a shallow layer in a porcelain or glass dish and heating in an air-bath for 1 hour at $105°$, cooling in a desiccator, and weighing. It is hardly necessary to take the precaution of drying in an inert atmosphere. These volatile constituents may be benzine, turpentine, benzene, etc.

Certain patents call for the use of sodium silicate (water glass) in the thickening of the oil. The alkaline nature of this substance would prohibit its use in the presence of blue dyes and Prussian blue. It will probably be found with the pigment, and is easily tested for by treating the pigment with boiling water, filtering off the undissolved material, and testing the filtrate with phenolphthaleïn. It can hardly be considered a desirable substance in printing inks.

ERRATA IN VOL. V.

INKS.

By PERCY H. WALKER.

For the estimation of tannin in writing inks reference may be made to F. W. Hinrichsen (*Die Untersuchung von Eisengallustinten*, page 65) and R. Kempf (*Untersuchung über Eisengallustinten*, Mitt. Königl. Materials Prüfungsamt, 1913, 451).

AMINES AND AMMONIUM BASES.

By W. A. DAVIS.

Estimation of Ammonia and Trimethylamine.

K. Budai[1] suggests the following method to estimate trimethylamine and ammonia when present together. The aqueous solution of the hydrochlorides of the two bases is treated with excess of formalin, previously made neutral to phenolphthaleïn and is then titrated with $N/10$ sodium hydroxide in presence of phenolphthaleïn; the number of c.c. used (x) corresponds with the *ammonia*.

$$4NH_4Cl + 6CH_2O + 4NaOH = (CH_2)_6N_4 + 10H_2O + 4NaCl$$
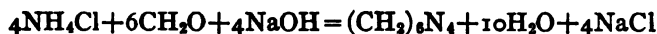
The solution is now diluted with a large quantity of water, made strongly acid with sulphuric acid and concentrated to one-third of its volume over a bare flame. The hexamethylenetetramine produced by the action of the formaldehyde on the ammonium salt is in this way hydrolysed into ammonia and formaldehyde. The solution is now made alkaline and distilled, the vapours being collected in excess of $N/10$ acid, the excess of acid subsequently remaining being titrated. From this titration the number of c.c. (y) of the $N/10$ alkali corresponding with the ammonia and trimethylamine is ascertained, and hence $y-x$ gives the value corresponding with the trimethylamine.

Hydrazine.

For the properties of hydrazine nitrate, see W. R. E. Hodgkinson.[2] This salt melts at 70° and may be kept at 100° for a long time without change. When heated at 200° *in vacuo* the gases evolved correspond with decomposition according to the equation: $4NH_2.NH_2, HNO_3 = 5N_2 + 2NO + 10H_2O$. Heated in a closed vessel the nitrate decomposes violently but under ordinary pressure merely burns rapidly. Contact with many oxi-dising agents, for example, permanganates, chromates, peroxides, causes inflammation. A solution of the nitrate does not act appreciably on zinc, cadmium and magnesium (which are, however, rapidly dissolved by cold saturated ammonium nitrate solution). Zinc, cadmium and copper added to the fused salt cause a flaming decomposition of the salt at slightly above 70°. Fragments of ordinary commercial cube cobalt or nickel added to the fused salt cause a violet explosion, but this behaviour is not shown by the

[1] *Zeit. physiol. Chem.*, 1913, **86**, 107.
[2] *J. Soc. Chem. Ind.*, 1913, **32**, 519.

same metals that have been melted and worked into wire, nor by these metals when prepared by reducing the oxides in hydrogen. In the latter case the metal is only slightly oxidised when added to the fused nitrate, which burns away rapidly.

According to Sommer[1] hydrazine may be estimated in presence of nitrous acid by titrating with iodine after adding bicarbonate, and the amount of nitrous acid may then be found by adding potassium iodide, displacing oxygen by carbon dioxide, adding dilute sulphuric acid and titrating with thiosulphate.

Estimation of Antipyrine.

W. O. Emery and S. Palkin[2] give the following methods of estimating antipyrine either alone or in presence of other substances.

I. *Alone or free from substances yielding a derivative capable of being extracted with chloroform.*

A quantity of the sample containing not more than 0.25 grm. of antipyrine is dissolved in 20 c.c. of water and treated with 5 c.c. of alcohol-free chloroform, 0.5 grm. of sodium hydrogen carbonate and a slight excess of iodine (15–20 c.c. of $N/5$ solution); after vigourously agitating at intervals during 5 minutes, the free iodine is removed by adding thiosulphate and the iodo-antipyrine extracted by shaking thrice with 25 c.c. of chloroform each time. The chloroform extract is washed with water, filtered, evaporated and the residue dried during 30 minutes at 110° and weighed. The weight multiplied by 0.5992 gives the quantity of antipyrine.

II. *When antipyrine is mixed with acetanilide, phenacetin, sulphonal or other substances which do not give an iodine-derivative insoluble in aqueous acid.*

The sample (containing not more than 0.25 grm. of antipyrine) is dissolved in 50 c.c. of water and shaken well with 20 c.c. of concentrated hydrochloric acid and 50–60 c.c. of $N/10$ iodine; after 3 hours, the clear liquid is decanted through a filter of glass wool and asbestos and the tarry precipitate of antipyrine periodide washed eight to nine times by decantation with 5% hydrochloric acid, and dissolved in 50 c.c. of methyl alcohol free from ethyl alcohol and acetone. The solution is treated with 5 c.c. of sodium hydrogen carbonate solution and 50 c.c. of water, shaken for 5 minutes, whereby the periodide is converted into iodo-antipyrine, the excess of iodine is removed by thiosulphate and the iodo-antipyrine extracted by shaking three times with 40 c.c. of chloroform each time and estimated as descibed in I.

Qualitative Tests for Antipyrine and Pyramidone.

According to Javillier[3] in a solution of antipyrine containing 0.7% of hydrochloric acid, silicotungstic acid produces a *white* precipitate of the

[1] *Zeit. anorgan. Chem.*, 1913, **83**, 119.
[2] *J. Ind. Eng. Chem.*, 1914, **6**, 1751.
[3] *Bull. Sci. Pharmacol.*, 1912, **19**, 70.

composition SiO_2, $12WO_3$, $2H_2O$, $4C_{11}H_{12}ON_2$, $7H_2O$, which loses $3\frac{1}{2}H_2O$ at 120°. A visible precipitate is produced in a solution containing only 1 part of antipyrine in 10,000. Under similar conditions in solutions of pyramidone containing 0.35% of hydrochloric acid, a *yellow* amorphous precipitate, SiO_2, $12WO_3$, $2H_2O$, $3C_{13}H_{17}ON_3$, $8H_2O$ is produced which loses the whole of its water at 120°.

According to Moulin[1] pyramidone produces a blue colouration with solutions of silver or mercury nitrate. No colouration is produced with pure nitric acid but if the acid contains nitrous acid, the colouration is obtained.

ERRATA IN VOL. VI.

Page 1, line 5 from bottom, "triamin-compounds," should read "triamino-compounds."
Page 4, line 2, "calcium hydroxide" should be "calcium oxide."
Page 36, first line of footnote, "levulinic" should be "lævulinic."
Page 49, line 2 from bottom, "halagen" should read "halogen."

[1] *Ann. Chim. Analyt*, 1912, **17**, 13.

ANILINE AND ITS ALLIES.

By S. S. SADTLER.

ESTIMATION OF ACETANILIDE AND PHENACETIN IN ADMIXTURE.

Phenacetin.—W. O. Emery[1] gives the following procedure: Into a small (50 c.c.) lipped Erlenmeyer flask introduce 0.2 grm. of the phenacetin-acetanilide mixture, add 2 c.c. of glacial acetic acid, heat gently over wire gauze to complete solution, then dilute with 40 c.c. of water previously warmed to 70°. Transfer the clear acetous liquid, by pouring and careful washing of the flask with two 10 c.c. portions of warm (40°) water, into a glass-stoppered, graduated 100 c.c. flask, into which have been previously run from a burette 25 c.c. of standard iodine, of a strength slightly above $N/5$ and warmed to 40°. Rotate the resulting menstruum to uniformity, the flask being closed meanwhile, then add 3 c.c. of concentrated hydrochloric acid, close flask again and continue rotation until copious crystallisation is apparent, then set the product aside to cool. If the ratio of phenacetin to acetanilide is equal to or greater than 1, crystalline scales will form almost immediately on adding the acid. As the proportion of acetanilide increases, however, the periodide is not only more inclined to maintain the liquid state, with the result that crystallisation becomes proportionately slower, but its separation also from the menstruum itself is in a measure retarded. In such cases, gentle agitation of the liquid or rotation of the flask in water warmed to 40° or less tends to promote the formation of crystals. When the contents of the flask have assumed the temperature of the room, fill up with water to within 2 to 3 c.c. of the mark, rotate to uniformity and allow to stand over night. Fill to the mark with water, mix thoroughly, then after standing 30 minutes withdraw a 50 c.c. aliquot of clear liquid by passing through a small (5.5 cm.) dry, closely fitted filter into a graduated 50 c.c. flask; the first 15 c.c. of the first runnings should be rejected, being received in any convenient container for eventual use later, along with additional filtrate, for the recovery of acetanilide. Transfer the 50 c.c. aliquot by pouring and washing to a 200 c.c. Erlenmeyer and titrate with $N/10$ sodium thiosulphate.

If the composition is considered of the insoluble addition product, $(C_2H_5O.C_6H_4NH.COCH_3)_2.HI.I_4$ formed in the foregoing separation, it will be noted that, for every molecule of phenacetin involved, 2 atoms of iodine are required; hence from a titrimetric standpoint, 1 atom of iodine is equivalent to $\frac{1}{2}$ mol. of phenacetin. If, therefore, the quantity

[1] *J. Ind. Eng. Chem.*, 1914, **6**, 665.

of iodine expended in the formation of insoluble periodide is ascertained as the result of such titration, the quantity of phenacetin thereby involved is readily calculated from the expression,

$$\text{phenacetin} = I\ (0.008890 \times N)$$

in which 0.008890 represents the quantity of phenacetin in 1 c.c. of an $N/10$ solution of this substance, N the normality of standard thiosulphate employed, while I represents the number of c.c. of such combination with phenacetin isolated as periodide.

The gravimetric estimation of phenacetin may, if desired, be effected substantially as follows: In the operation of filtering off the periodide, the latter is collected on the filter and washed with 10 to 15 c.c. of standard iodine solution, preferably by suction, then transferred, together with the filter (likewise any particles of precipitate which may remain in the graduated flask) to a separating funnel, using for the purpose not more than 50 c.c. of water. After discharging both free and added iodine with a few small crystals of sodium sulphite, the liquid is extracted with three 50 c.c. portions of chloroform, each portion being subsequently washed in a second separating funnel with 5 c.c. of water. After washing and clearing, the solvent is passed through a small (5.5 cm.) dry filter into a 200 c.c. Erlenmeyer, most of the chloroform removed by distillation, and the residual 5 to 10 c.c. are transferred by pouring and washing with fresh solvent into a small tared beaker or crystallising dish. The solution is evaporated to dryness on the steam-bath, and the residue cooled and weighed.

Acetanilide.—Should the combined weight of the phenacetin-acetanilide mixture be known, that of the latter constituent can be determined by difference, or, if necessary, estimated directly from a second aliquot of filtrate from the phenacetin-periodide.

To this end, transfer to a separating funnel by means of a pipette 25 to 30 c.c. of the clear liquid, decolourise with sufficient solid sodium sulphite, add solid hydrogen sodium carbonate in slight excess, follow with 1 to 2 drops of acetic anhydride, then extract with three 60 c.c. portions of chloroform, passing the solvent when cleared through a small, dry filter into a 200 c.c. Erlenmeyer flask; the chloroform is distilled off by the aid of gentle heat until the volume is about 20 c.c. Now add 10 c.c. of dilute sulphuric acid (1 c.c. of concentrated acid to 10 c.c. of water) and digest the product on the steam-bath until the aqueous residue has been reduced to one-half, add 20 c.c. of water and continue the digestion 1 hour, add a second 20 c.c. portion of water and 10 c.c. of concentrated hydrochloric acid, then titrate very slowly drop by drop, with standard potassium bromide-bromate (1 c.c. of which is equivalent to 5 to 10 mg. of acetanilide), until a faint yellow colouration persists. While adding this reagent, the flask should be rotated sufficiently to agglomerate the precipitated tribromaniline and thus clarify the supernatant liquid. The number of c.c. of standard bromide solution required

to complete the precipitation, multiplied by the value of 1 c.c. in terms of acetanilide, will give the quantity of this substance present in the aliquot taken.

ERRATA IN VOL. VI.

Page 57, line 3 from bottom, for "sulphate" read "sulphide." Line 2 from bottom, for "as" read "at."

Page 58, line 12, for "if" read "it."

Page 59, line 11 from bottom, for "hydrochlorate" read "hydrochloride."

Page 60, line 18 from bottom, for "or pure aniline hydrochlorate" read "of pure aniline hydrochloride."

Page 60, line 3 from bottom, for "centimetres" read "cubic centimetres."

Page 63, line 5 from bottom, formula for toludines should be $C_7H_7.NH_2$ not $C_7H_7.HN_2$.

Page 66. The top paragraph, from "p-Toluidine is produced" to "recalling that of aniline "should be transferred to page 65, and inserted after the paragraph dealing with meta-toluidine."

Page 68, line 16, for "phosphates" read "phosphoric acid." Line 23 for "hydrochlorates" read "hydrochlorides."

Page 69, line 1, for "the" read "then."

Page 71, footnote line 4, for "v-oxylidine" read "v-o-xylidine."

Page 75, line 8 from bottom, for "hydrochlorate" read "hydrochloride."

Page 76, line 1 for "which" read "while."

Page 82, line 12 from bottom, for "Acet-phenethidine" read "acet-phenetidine."

Page 85, footnote, for "amido" read "amino," and for "levorotatory" read "lævorotatory."

Page 86, line 12, for "phenylformamid" read "phenylformamide."

Page 86, line 16, for "anesthetic" read "anæsthetic." Line 17, for "Gallanid" read "Gallanide."

Page 89, in footnote delete lines 2 to 4, which duplicate the text.

Page 90, line 13 from bottom, for "Vol. 4" read "Vol. 5."

Page 92, line 4 below table for "methylamine" read "methylaniline."

Page 93, line 1 for "acetylisation" read "acetylation." Line 6, delete "Recognition of in the presence." Line 7, after bracket add "as follows." Line 10 and line 13, for "CL" read "Cl." Second line from bottom, for "aliquod read "aliquot."

Page 96, line 3; delete the whole of this line. Line 17, for equation given, read $(C_6H_5)_2NH + 8Br = (C_6H_3Br_2)_2NH + 4HBr$.

Page 97, line 3, delete "J. W. G."

Page 97, line 2 from bottom for "p-minophenol" read "p-aminophenol."

Page 98, line 12 for "anisols" read "anisoles." Line 13, for "phenethidine" read "phenetidine." Line 13, for "aminophenatols" read "aminophenetoles." Line 15 for "Anisol" read "Anisole;" "Phenatol" read "Phenetole." Line 17, for "metacetin" read "methacetin." In table, division 4 from bottom, for "phenethidine" read "phenetidine."

Page 99, line 13 and line 19 for "acetphenetidin" read "acetphenetidine."

Page 100, line 15 and line 9 from bottom, for "phenetidin" read "phenetidine." Line 6 from bottom for "diaminophenatols" read "diaminophenetoles."

Page 102, line 3 for "Million's" read "Millon's."

Page 104, line 21 from bottom, for "phenethidine" read "phenetidine." Line 16 from bottom, for "phenetidin" read "phenetidine." Line 12 from bottom, for "amidin" read "amidine." Line 9 from bottom, for "anesthetic" read "anæsthetic."

Page 107, line 14 for "Diamiotoluenes" read "Diaminotoluenes."

Page 109, line 18 from bottom, for "Orthotoluidine" read "Orthotolidine." Line 5 from bottom, for "Toluidine" read "Tolidine."

THE NAPHTYLAMINES AND THEIR ALLIES.

By EDWARD HORTON.

PYRIDINE.

Detection.—To detect pyridine in "Liquor Ammonii caustici" Kunze-Krause[1] recommends that 11 or 12 c.c. of the liquor contained in a test-tube should be neutralised gradually, but as quickly as possible, by adding 5 grm. of powdered tartaric or citric acid, the mixture being constantly stirred. Before and after the addition of the final quantity of acid, the hot liquid is thoroughly shaken and immediately smelt. When the smell of ammonia has disappeared, it should be odourless. Very small quantities of pyridine give a recognisable smell.

Wöhlk[2] detects pyridine in ammonium salts by grinding about 0.5 grm. of the latter in a mortar with 1 grm. of borax. If pyridine be present it is immediately recognised by its characteristic odour.

Estimation.—In the analysis of mixtures of ammonia and pyridine, Delépine and Sornet[3] remove the ammonia by Gerresheim's method,[4] by precipitating it from solution in hydrochloric acid with mercuric chloride in the presence of sodium carbonate and sodium hydroxide. The pyridine is distilled out of the filtrate and weighed either as the aurichloride or the platinichloride.

Bayer[5] states that in titrating pyridine in ammonium salts, ferric thiocyanate is a more satisfactory indicator than either methyl orange or ferric chloride. The mixed bases are acidified with $N/10$ hydrochloric acid, treated with a drop of ferric chloride solution, and one of ammonium thiocyanate solution and titrated with $N/10$ sodium hydroxide solution until the brownish-red colour is destroyed. The bases can be partially separated by distilling the neutral solution of the mixed salts, when the pyridine passes over together with a very small amount of ammonia. The latter is titrated with $N/10$ hydrochloric acid in the presence of litmus, then excess of acid is added and the pyridine estimated as above.

A method of estimating pyridine in ammonia water, which depends on

[1] *Apoth. Zeit.*, 1910, 25, 87.
[2] *Ber. deut. Pharm. Ges.*, 1912, 22, 825.
[3] *Bull. Soc. Chim.*, 1911 [iv]. 9, 706.
[4] *Annalen*, 1879, 195, 373.
[5] *J. Gasbel.*, 1912, 55, 513.

the destruction of the ammonia with sodium hypobromite has been described by Houghton.[1] 100 c.c. of the ammonia water are diluted with 150 c.c. of distilled water in a litre flask and a few drops of methyl orange solution added. The flask is cooled in running water and the liquid neutralised with dilute sulphuric acid (1 : 3) and made slightly acid, then 5 c.c. of $N/1$ NaOH solution are added and the liquid distilled (if the proportion of pyridine present is high, either a smaller volume of ammonia water must be used or more than 5 c.c. of $N/1$ alkali solution added). To destroy the ammonia, the distillate is treated with 100 c.c. of a sodium hypobromite solution (prepared by dissolving 100 grm. of sodium hydroxide in a litre of water and adding 25 grm. of bromine) and shaken until no more gas is evolved. The unchanged pyridine is then distilled into excess of $N/10$ acid, the excess being titrated with $N/10$ alkali using methyl-orange as indicator (1 c.c. $N/10$ acid is equivalent to 0.0079 grm. pyridine). A more rapid modification of the same method is described by Baessler,[2] in which the vapour from the slightly alkaline solution of ammonium and pyridine sulphates is made to pass through a sodium hypobromite solution, the vapour from which (containing pyridine only) is collected in the $N/10$ acid.

It is stated by Fincke[3] that a proportion of the spirit used in the manufacture of vinegar has been denatured with pyridine, which accordingly occurs in the resulting product. For its estimation therein Fincke holds that Lunge's method (*Chem. Techn. Unters. Methoden*, 5th Ed., Berlin, 1905, **3**, 583) is inaccurate, and recommends that described by Houghton (*loc. cit.*).

Pyrrole.

Herzfeld describes[4] the following simple test for pyrrole, which has the advantage of distinguishing it from indole. An indole solution when treated with solutions of sodium hydroxide and sodium nitroprusside acquires a violet blue colour, which on addition of acetic acid becomes blue. Under the same conditions a pyrrole solution gives a brownish-red colour, which, unlike the violet blue of indole, can be extracted with chloroform. With a solution containing both indole and pyrrole after shaking with chloroform, a liquid is obtained which consists of a blue upper layer and a brownish-red lower layer.

ERRATUM IN VOL. VI.

Page 121, headline, for "sulphuric acids"read "sulphonic acids."

[1] *J. Ind. Eng. Chem.*, 1909, **1**, 698.
[2] *J. Gasbel.*, 1912, **55**, 905.
[3] *Zeitschr. Nahr. Genussm.*, 1911, **21**, 655.
[4] *Biochem. Zeit.*, 1913, **56**, 82.

THE VEGETABLE ALKALOIDS.

By THOMAS A. HENRY, D. Sc.

Since the date of the previous article a great deal of work on alkaloids has been published but it is mainly concerned either with the determination of the constitution of well-known alkaloids, or with the isolation and characterisation of new alkaloids. Comparatively few new facts which affect alkaloids as a class have been recorded.

Formation and Function of Alkaloids in Plants.—In the original article it was pointed out that two views are held as to the mode of formation of alkaloids in plants. Some authorities believe that they are products of direct synthesis, whilst others regard them as formed, directly or indirectly by the decomposition of proteïns first formed. It seems to be clearly established that where alkaloids do not occur in the seeds of plants, which normally contain them, they are produced almost as soon as the seeds germinate: thus Torquati[1] found that ungerminated barley is free from hordenine but that the base is formed immediately on germination and steadily increases in amount until the fourth day when it reaches a maximum and then gradually diminishes. Similarly Kerbosch[2] found that although the seed of the opium-poppy contains only a trace of narcotine and amorphous alkaloids, the amount of narcotine increases on germination and the other characteristic opium alkaloids appear in the order, codeïne, morphine, papaverine, narceïne, and thebaine. This increase occurs even when the seeds are germinated in a nitrogen-free atmosphere, whence it appears that the narcotine and other alkaloids are formed at the expense of protein. de Plato's observation that tobacco seed is free from cyanogenetic glucosides and alkaloids but contains allantoin is also of interest in this connection.[3] Ciamician and Ravenna have applied their inoculation method to tobacco[4] and find that whilst inoculation with asparagine or dextrose causes an increased production of alkaloids, the application of pyridine, piperidine, or pyrrolecarboxylic acid does not, in spite of the fact that nicotine, the chief tobacco alkaloid is N-methyl-α-β-pyridylpyrrolidine. The results of all this work still leave the question of the mode of formation of alkaloids open, though on the whole they may be said to lend support to the view that alkaloids are formed from the decomposition products of proteins or at least from similar amino-compounds.

[1] *Arch. Farmacol. sperim.*, 1910, 10, 62 and 97.
[2] *Pharm. Weekblad*, 1910, 47, 1062, 1081, 1106.
[3] *Stas. sper. Agrar. ital.*, 1910, 43, 79.
[4] *Atti. R. Accad. Lincei*, 1911 [v], 20, i, 614.

Closely allied to this subject is that of the function of alkaloids in plants. Probably no one now seriously holds the view that alkaloids are a means of protection for the plants which produce them. The view most widely accepted is that they are ultimate products of metabolism and of no further use to the plant. Apart from the facts supporting this view referred to in the original article, van Leersum[1] has disproved Lotsy's contention that in cinchona amorphous alkaloids are produced in the leaves under the stimulus of light and air, and are then transported to the stem where they are elaborated into quinine and cinchonine. According to van Leersum the two latter alkaloids are formed in the leaves and the amount in these members remains constant even after the leaves have fallen from the tree. Tunmann[2] has investigated the behaviour of the alkaloids in nux vomica seeds and shown that there is no ground for the view that in this case the alkaloids are used by the embryonic plant.

The most useful evidence recently brought forward for the opposite view, viz., that alkaloids are assimilation products destined to serve the purposes of nutrition in the plant is that produced by Schmidt[3] and his collaborators, Müller[4] and Klee.[5] Müller in particular has shown that in the opium-poppy grown under natural conditions, alkaloids begin to appear in the plant 14 days after germination; the amount increases until reserve albumin begins to accumulate in the seeds, when it begins to diminish in the leaves, fruits and stems though it never disappears entirely from these organs, whence Müller concludes that in this species at least, the alkaloids are used in producing protein during the ripening of the seed. A certain amount of support for this view is also afforded by Schloesing's observation[6] that a normally grown tobacco plant contains much less nicotine than one from which the leaves have been partially removed.

Properties of the Alkaloids.—It was pointed out in the original article that most alkaloids are optically active and that certain alkaloids (e.g., nicotine and aconitine) exhibit a rotatory power opposite in sign to that of their salts. Carr and Reynolds have shown[7] that though comparatively few alkaloids show a change in the sign of the rotatory power on neutralisation, a change in the value of the rotatory power is not uncommon especially among the tertiary alkaloids, even when both determinations are made in the same dissociating solvent; thus l-hyoscine in water has $[\alpha]_D - 28.0°$ as base and $[\alpha]_D - 32.8°$ in the form of a salt. Quinine in 50% alcohol has $[\alpha]_D - 170.5°$ as base and $[\alpha]_D - 262.1°$ as salt. It is well known that the nature of the solvent and the concentration of the solution also affect the rotatory power of certain alkaloids and the same authors record new instances of the effect of these factors; thus hydrastine in the form of hydrochloride has $[\alpha]_D + 158°$

[1] Proc. K. Akad. Wetensch, Amsterdam, 1910, 13, 210.
[2] Arch. Pharm., 1910, 248, 644.
[3] Ber. deut. pharm. Ges., 1914, 24, 35.
[4] Arch. Pharm., 1914, 252, 280.
[5] Ibid., 211.
[6] Compt. rend., 1910, 151, 23.
[7] Trans. Chem. Soc., 1910, 97, 1328.

in water, and + 197° in 50% alcohol. The same alkaloid, as free base, is strongly dextrorotatory in 50% alcohol, optically inactive in 95% alcohol and markedly lævorotatory in 100% alcohol.

General Precipitants for Alkaloids.—A certain number of new precipitants for alkaloids have been described but none of these appears at present to offer any advantages over those already in common use.

Silicotungstic acid, first used by R. Godeffroy as a precipitant for alkaloids in slightly acid solution has acquired some importance recently owing to its having been applied to the estimation of nicotine, atropine, and other alkaloids, especially by Bertrand and Javillier.[1]

ERRATA IN VOL. VI.

Page 167, line 5 from bottom, insert after the bracket "are small in number" and delete the next nine words.

Page 168, line 7, replace "No alkaloid has" by "Only two alkaloids have."

Page 172, line 11 after "weight of" insert "magnesia or."

Page 172, line 4 from bottom, for "South Wark" read "Southwark."

Page 173, line 3 from bottom, between "the" and "ground" insert "dry."

Page 174, line 15 from bottom, after "residue" delete "extracted" and insert "thoroughly mixed."

Page 177, line 17 from bottom; delete this and two next lines.

Page 181, line 12 from bottom, after "phenolphthaleïn" insert (cf. Elvove, *J. Amer. Chem. Soc.*, 1910, **32**, 132).

Page 182, bottom line, after "sparteine" insert "nicotine, pilocarpine."

Page 183, line 3 for "iodesin" read "iodeosin."

Page 184, line 2 from bottom, add at end "methods involving sublimation have been suggested recently for the detection or estimation of certain alkaloids. (Burmann, *Bull. Soc. chim.*, 1910 (iv), **7**, 239; Eder, *Schweis. Woch. Chem. Pharm.*, 1913, **51**, 228, 241, 253; Tunmann, *Pharm. Zentr.-H.*, 1913, **54**, 1065)."

Page 199, line 19, delete "and papaverine."

Page 201, line 7 from bottom, after "with" add "hydrogen peroxide."

Page 202, bottom line, for "produces" read "produce."

Page 203, line 7 from bottom, delete "and codeine."

Page 206, line 14 from bottom, delete all after "coca alkaloids."

[1] *Compt. rend.*, 1899, **128**, 742; *Bull. Soc. chim.*, 1909 [iv], **5**, 241; *Bull. Sci. Pharm.*, 1909, **16**, 315; 1910, **17**, 629; *U. S. Dept. Agric., Chem. Div. Bulletin No. 101*, of 1910; *Ann. Chim. Anal.*, 1911, **16**, 251; *Gazette* 1913, **43**, ii, 482 and *Schweis. Woch. Chem. Pharm.*, 1913, **51**, 761.

VOLATILE ALKALOIDS.

By FRANK O. TAYLOR.

Areca Alkaloids (Compare Vol. VI, pp. 208–211).—**Arecolidine.**—H. Emde[1] has isolated from the mother liquors obtained in the preparation of arecoline hydrobromide minute quantities of a new alkaloid to which he gives the name *arecolidine* and assigns the formula $C_8H_{13}O_2N$, its constitution being probably $CH:CH.C(OCH_3):C(OCH_3):CH_2·NCH_3$. Separation is effected by the continued recrystallisation of the hydrochlorides. From the pure salt, arecolidine is liberated even by mild alkalis, such as magnesium carbonate. The base is easily soluble in water, alcohol, ether and acetone and on evaporation of the aqueous solution remains as a pleasantly odorous, thick oil. From anhydrous ether the base is obtained in brilliant needles, m.p. 105°, but sublimation raises this to 110°. The crystalline base is very hygroscopic. *Arecolidine hydrochloride*, $(C_8H_{13}O_2N.HCL-H_2O)$ crystallises from 99.5% alcohol in hygroscopic prisms m.p. 95°–98°. At 100° it loses its water and at 250° decomposes.

Tunmann[2] has found that the alkaloids are contained in the cells of the endosperm and not in the veiny tissue. He devised a method for the microscopical detection of the alkaloids in sections of the nuts, as follows:

Prepare a filtered solution of 0.1 grm. of picrolonic acid in a mixture of 3 c.c. alcohol and 2 c.c. water. Drop the prepared section into some of this solution on a slide, put on a cover glass, seal in place with wax and let stand. Within 2 days characteristic sphæro-crystals are formed inside the cellular tissue.

From the dry powder characteristic fatty acid crystals may be sublimed. In the original article both kinds of crystals are illustrated.

An acceptable method of assay is that of the Swiss Pharmacopœia, which requires a standard of 0.5% arecoline. To 12 grm. of powdered areca nuts contained in a tightly stoppered flask add 120 c.c. of ether and allow to macerate for 15 minutes with frequent shaking. Now add 5 c.c. of 10% ammonia and continue the digestion for an hour with frequent vigorous shaking, preferably continuously in an automatic shaker. Let stand until the supernatant ethereal solution is clear, and then decant 100 c.c. through a pledget of cotton into another flask, evaporate carefully to remove all ether, dissolve the residue in 5 c.c. of alcohol, add 10 c.c. of water, 3 drops of hæmatoxylin solution and 30 c.c. of ether, and titrate with $N/10$ hydrochloric acid till the

[1] *Apoth. Zeit.*, 1915, 30, 240.
[2] *Pharm. Post*, 1911, 44, 703.

colour of the aqueous layer begins to change; now add 30 c.c. of water and carefully finish the titration to a yellowish end-point, vigorously shaking. During this treatment at least 3.3 c.c. of $N/10$ acid should be consumed, equal to not less than 0.5% arecoline in the drug. The same process may be applied to fluid and solid extracts by evaporating equivalent amounts to dryness on washed sawdust, taking this in place of the powdered drug.

By this process Puckner[1] found 0.44% and 0.52% in two samples.

Conium Alkaloids (Vol. VI, pp. 211–223).—Since the publication of Vol. VI there has been no work on these alkaloids having any important bearing on analytical processes.

Neogi[2] in the course of work on nitrites of various bases produced *coniinium nitrite* by repeated distillation *in vacuo* of a mixture of coniine hydrochloride and an alkali nitrite. This substance forms deliquescent, fibrous crystals which melt at 84° and sublime *in vacuo*. When heated to 120° under atmospheric pressure it forms *nitrosoconiine*. He also obtained *coniine methonitrite*, $C_8H_{17}N$, $CH_3.NO_3$, as a viscous reddish-yellow liquid.

A method for the assay of conium that in several ways is preferable to others described in Vol. VI is one worked out in Parke, Davis & Co.'s laboratory.

To 10 grm. of the finely powdered drug add 5 c.c. of 5% hydrochloric acid and 50 c.c. of petroleum ether (ligroin); shake well for 5 minutes and let settle. Decant the petroleum ether and repeat the operation. After the second washing (which operation removes the fat that otherwise interferes with the assay) remove the petroleum ether fully by decantation and evaporation of the last portions in a current of air. Now add to the still moist drug, in a tightly stoppered flask, 80 c.c. of petroleum ether and 1 grm. of potassium carbonate; shake well for several minutes and let stand over night. Transfer exactly 40 c.c., representing 5 grm. of the drug, through a pledget of cotton to another flask, add 10 c.c. of $N/10$ acid, evaporate the petroleum ether at a low temperature, preferably under a current of warm air, and titrate the excess acid with $N/50$ alkali. Each c.c. of $N/10$ acid consumed equals 0.0127 grm. coniine.

The fluid extract may be treated directly by the same process without previously evaporating it.

Lobeline (Vol. VI, pp. 223–225).—Owing to its comparatively restricted use, lobelia and its alkaloids have received practically no attention during the last 3 years.

Lupine Alkaloids (Vol. VI, pp. 225–229).—The high protein content of the lupines makes them especially useful as fodder for animals were it not for the marked bitter taste due to the alkaloids. This bitterness may be removed by washing with water and drying, or preferably by steaming, after which the plants are mashed between hot rollers to produce the so-called

[1] *Rep. Lab. Am. Med. Assoc.*, 4, 119.
[2] *Trans.*, 1912, 101, 1608.

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"lupine flakes." Assays of these by Stutzer and Gay[1] gave the following results·

Crude protein	37.06 %	31.93 %
Crude fat	4.20 %	3.89 %
Nitrogen free extractive	41.48 %	39.10 %
Crude fibre	13.46 %	7.02 %
Digestible protein	31.55 %	

See also Honcamp.[2]

Dextro-lupanine (VI, p. 228) has been compared with oxysparteine, by Beckel,[3] who finds that these alkaloids are not identical since they give different halogen alkyl derivatives. He also[4] fails to verify the work of Soldaini and of Davis who obtained two new bases having the formulæ $C_8H_{16}ON$ and $C_7H_{11}O_2N$, by the action of bromine on *d*-lupanine hydrochloride in alcoholic or acetic solution. By similar treatment Beckel claims the formation of *ethoxylupanine dihydrobromide*, $C_{15}H_{22}ON_2.OC_2H_5,2HBr$, which forms colourless needles from hot alcohol, m. p. 227–228°, very soluble in water but difficultly so in alcohol, $[\alpha]_D = -129.4°$ in 0.8% to 3.1% aqueous solution, or in 1.7% alcohol. Ethoxylupanine also forms a *dihydroiodide* on boiling the dihydrobromide with hydriodic acid; it forms needles from alcohol, m. p. 221–222°, $[\alpha]_D = -106.2°$ (0.2627 grm. in water to make 19.66 c.c.). A *thiocyanate* has been described, crystallising in needles, easily soluble in water and slightly soluble in alcohol, m. p. 172–174° and $[\alpha]_D = -133.4°$ (0.3983 grm. in water to 18.66 c.c.).

d-Lupanine dihydrobromide, $C_{15}H_{24}ON_2,2HBr,H_2O$, forms needles, m. p. 188°–189°, $[\alpha]_D = +45.9°$, which on drying lose both water and hydrogen bromide.

Di Palma[5] describes the action of heat up to 300° on *d*-lupanine, stating that after the evolution of alkaline gases smelling of pyridine there can be isolated from the residue a base having the formula $C_{15}H_{22}N_2$ which gives a *hydrochloride* melting with decomposition at 165°; a *platinichloride* + $5H_2O$, m. p. (when dried) 117°–119°, and an *aurichloride* melting with decomposition at 160°–165°.

Inactive lupanine was found by Beckel (*loc. cit.*) in the seeds of *Lupinus angustifolius*, as the chief constituent of alkaloids present to the extent of 0.9% to 1.2% and in the pods about one-fifth as much. A crude alkaloid from the seed prepared by extraction with alcoholic hydrochloric acid consisted of about 38% of *hydroxylupanine*, which may possibly have been formed during the extraction process. He describes an abnormal aurichloride and platinichloride of the *dl*-lupanine methochloride.

Piturine.—The work of Rothera[6] has verified and supplemented that of Langley and Dickinson and of Petit, with the result that piturine is found to be identical with nicotine, and not a distinct alkaloid.

[1] *Landw. Vers. Sta.*, 1913, **78–80**, 219; *Chem. Abst.*, 1913, **7**, 2811.
[2] *Wiener. Landw. Ztg.*, **63**, 315.
[3] *Arch. Pharm.*, 1911, **249**, 329.
[4] *Ibid.*, **250**, 691.
[5] *Giorn. Farm. Chim.*, 1913, **61**, 151.
[6] *Bio-chem. J.*, 1910, **5**, 193.

Pomegranate Alkaloids.—No new information of analytical interest has appeared since the publication of Vol. VI.

Sparteine and other Spartium Alkaloids (Vol. VI, pp. 232–235).— During the past few years considerable work has been done in attempts to elucidate the exact constitution of sparteine, but not much of it is of analytical interest. For theoretical considerations and a mass of data sustaining them see Moureu and Valeur;[1] Germain,[2] and Corriez.[3]

Corriez describes the following new salts of sparteine:[4]

Dichlorate, $C_{15}H_{26}N_2,2HClO_3$, decomposing explosively at 147° without melting; $[\alpha]_D = -23.12°$.

Monochlorate, $C_{15}H_{26}N_2,HClO_3$, m. p. 139°–140°, exploding at 200°– 205°; $[\alpha]_D = -16.3°$.

Diperchlorate, $C_{15}H_{26}N_2,2HClO_4,2H_2O$, m. p. 78°, but if anhydrous at about 265°, exploding at 300°, $[\alpha]_D = -17.3°$.

Monoperchlorate, $C_{15}H_{26}N_2,HClO_4$, m. p. 171°, decomposing at 300°; $[\alpha]_D = -16.3°$.

Dichromate, $C_{15}H_{26}N_2,H_2Cr_2O_7$, darkens in the light and decomposes on heating, soluble in 50 parts of water.

Salicylate, $C_{15}H_{26}N_2,2C_7H_6O_3,H_2O$, m. p. 78°; $[\alpha]_D = -9.42$.

He also describes[5] the *perbromide* made by the action of bromine in fuming hydrobromic acid on sparteine dissolved in the same acid; this interaction may be used to detect sparteine. By adding 2 c.c. of the aqueous solution to be tested to an equal volume of hydrobromic acid containing a small quantity of free bromine (1 or 2%), the presence of as little as 0.01 mg. of sparteine will show a yellowish precipitate. (The perbromide melts at 193°).

In an attempt to produce habituation to sparteine, Dolencourt[6] demonstrated by intramuscular injections that the lethal dose of sparteine sulphate in guinea-pigs is 0.0066 grm. per 100 grm. of body weight, which, if the same held true for human beings would be the extraordinary amount of 4.5 grm. (about 70 grains) per 150 pounds.

Genisteine, $C_{15}H_{28}N_2$, is a new volatile, crystalline base of low melting point discovered by Valeur[7] in the mother liquors from the crystallisation of sparteine sulphate. It melts at 60.5° and boils at 177°–178° (not corr.). Potassium permanganate in sulphuric acid does not oxidise it. The following salts are described:

Hydrate, $C_{15}H_{28}N_2,H_2O$, m. p. 117°; $[\alpha]_D$ (4% solution in alcohol) = − 52.34°. *Picrate,* m. p. 215° (with decomposition). *Platinichloride,*

[1] *Comp. rend.,* 1912, **154**, 161 and 309; and chiefly *Ann. Chim. phys.,* 1912, **27**, 245–391 which is a résumé of some 10 years' work previously published in various journals.
[2] *Gazzetta,* 1912, **42**, i, 447.
[3] *Bull. sci. pharmacol,* 1912, **19**, 468, 527, 533, 620.
[4] *Ibid.,* **19**, 468.
[5] *Ibid.,* **19**, 533.
[6] *Compt. rend. soc. biol.,* 1913, **74**, 801.
[7] *J. pharm. chim.,* 1913, **8**, 573.

crystallising with $2H_2O$ which it loses at $110°$; blackens without melting at $235°$.

ERRATA IN VOL. VI.

Page 207, line 13, for "Lupinined" read "Lupinidine."

Page 210, line 30, for "Jahrs" read "Jahns."

Page 211, lines 3 and 21, for "*Jahr's*" read "Jahns."

Page 212, in table, for "methyl-conine" read "methyl-coniine."

Pages 213, 215, 217, 219, 221 title, for "ARECA OR BETEL-NUT" read "CONIUM OR HEMLOCK."

Page 233, line 17, for "sparteine" read "sparteine sulphate."

ESTIMATION OF NICOTINE.

BY R. W. TONKIN.

The frequency with which the estimation of nicotine is hindered by the presence of ammonia or substances which yield it on treatment with alkalies has led to the introduction of polarimetric methods.

Tóth[1] modifies his method, given in Vol. VI of this work (page 240) by substituting xylene for the extracting agent and calculates the amount of nicotine by observing the rotation of the fluid, the specific rotation of nicotine in a dilute xylene solution being +173°.

König[2] in the case of tobacco extracts, dilutes 10 c.c. with 5 c.c. of water, adds 2 c.c. of 1 : 1 sodium hydroxide solution and some glass beads, and extracts the nicotine by shaking for 2 hours with 50 c.c. of toluene. Xylene can be substituted for toluene. The nicotine is calculated from the observed rotation. The nicotine can then be titrated by shaking with water and standard acid using iodeosin as indicator as usual.

Bertrand and Javillier[3] heat 12 grm. of tobacco in a flask with reflux condenser for an hour with 300 c.c. of 0.5% hydrochloric acid; after cooling, the contents of the flask are filtered, and 250 c.c. of the filtrate are treated with a 10–20% solution of potassium silico-tungstate in slight excess. If nicotine is present in any quantity, a precipitate forms rapidly, but it is advisable to let the mixture stand for a day or two to ensure that the whole is precipitated. The composition of the precipitate dried at 130° is expressed by the formula. [$12WO_3, SiO_2, 2H_2O : 2C_{10}H_{14}N_2$]. The composition is not quite constant when thus dried, but the nicotine may be calculated from the weight of the residue left on ignition by using the factor 0.1071.

As many other bodies give a similar precipitate with the silico-tungstate solution, it is preferable to distil off the nicotine and titrate it in the distillate. The precipitate is introduced in a flask with 125 c.c. of water and some magnesium oxide and 100 c.c. distilled over, when the whole of the nicotine will have been expelled.

The method is a good one for estimating small amounts of nicotine when time is no object.

Harrison and Self[4] give the following: An amount of tobacco or extract is taken which will yield about 0.5 grm. nicotine, mixed with lime and water

[1] *Chem. Zeit.*, 1911, 35, 926.
[2] *Chem. Zeit.*, 1911, 35, 521.
[3] *Bull. Soc. Chim.*, 1909 [iv], 5, 241.
[4] *Pharm. J.*, 1912, 34, 718.

and steam-distilled till the whole of the volatile bases have passed over; these are absorbed in a measured amount of standard acid and the total bases determined by titrating back. 10 c.c. of the acid are then added and the liquid evaporated to 50 c.c. (no ammonia is lost by this process) and iodine dissolved in potassium iodide solution is added to precipitate the nicotine. The iodine should be 10 times that required to combine with the total bases calculated as nicotine. The solution is made up to 100 c.c. and filtered; 75 c.c. of the filtrate are taken and the excess of iodine removed with sodium thiosulphate. After adding 25 c.c. of 10% sodium hydroxide solution, the ammonia is distilled off into an excess of standard acid, and determined by titrating back. The difference between the total bases and the ammonia is returned as nicotine.

A large excess of alkali is necessary in the last distillation as otherwise the liquid may become acid, probably owing to the decomposition of the tetra-thionate formed while removing the excess of iodine.

R. Spallino[1] publishes a method based on the fact that nicotine in alcoholic solution acts as a mono-acidic base towards picric acid, whereas in aqueous solution it is diacidic.

The liquid containing nicotine is treated with an excess of picric acid solution of known strength (9 mg. per c.c.), and the mixture made up to 1 litre with alcohol; it is then divided into 2 equal parts which are evaporated to dryness. In one case the residue is dissolved in alcohol and titrated with $N/20$ barium hydroxide. The other portion is rubbed up with water, made up to 100 c.c. filtered to remove undissolved picrate, and 75 c.c. are titrated as before. The difference between the results of the two titrations, after allowing for 25 c.c. not used in the second case, gives the amount of picric acid which could combine with half the nicotine present. The method gives concordant figures for analyses of ammoniacal distillates from tobacco.

For a polarimetric method of estimating nicotine in tobacco extracts see Degrazia.[2]

ERRATA IN VOL VI.

Page 237, line 9 for "nicotyrin, l-methyl-" read "nictotyrine (l-methyl;" and insert bracket in line 10 after pyrrole.

Page 242, line 2 for "thesk in" read "the skin."

[1] *Gazzetta*, 1913, 43, ii, p. 493.
[2] *Fachl. Mitt. Oesterr. Tabakregie*, 1910, 87, and 149; *Chem. Zentr.*, 1911, 1, 1085 and 1086.

ACONITE ALKALOIDS.

By FRANCIS H. CARR.

The Alkaloids of Aconitum Napellus.—Our knowledge of the constitution of aconitine—the chief of the aconite alkaloids—has made some very slight progress since the publication of Vol. VI. This is due principally to work on its oxidation. It has been shown by one worker[1] that when aconitine is oxidised with permanganate in acid solution, acetaldehyde and a neutral substance, *oxonitin*, $C_{23}H_{29}O_9N$, m. p. 274°, are produced. Another worker,[2] while confirming this, has expressed the view that when neutral permanganate is employed, a substance of similar properties, but having the formula $C_{24}H_{29}O_9N$, is produced. It appears likely that further investigation will establish the identity of these two compounds.[3] Oxonitin retains three methoxyl groups as well as the N-methyl, benzoyl and acetyl groups of aconitine. It may be represented:

$$C_{10}H_9O_2N \begin{cases} CH_3 \\ O.Ac \\ O.Bz \\ (O.CH_3)_3 \end{cases}$$
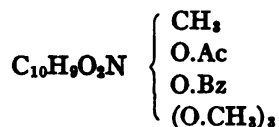
On hydrolysis, acetic and benzoic acids are split off and an alkaloid, soluble in ether, remains.

By the action of nitric acid a yellow crystalline substance $C_{22}H_{26}O_{11}N_2$,[4] m. p. 205°, results. It contains a nitroso and two carboxyl groups.

A substance $C_{22}H_{43}O_9N$ prepared by heating aconitine in a similar manner to that employed by Dunstan and Carr for pyraconitine has been described by Schulze and Liebner.[5] Its properties, however, differ considerably from those of pyraconitine and it would appear to be a new alkaloid. The new base melts at 171° and has $[\alpha]_D$ in alcohol −112°. Pyraconitine melts at 167°-168° and its solution in alcohol is optically inactive. The *hydrobromide* of the new base melts at 240° and has $[\alpha]_D$ in water −106°. Pyraconitine hydrobromide melts at 280°, and has $[\alpha]_D$ in water −47°. The *hydrochloride* of the new base melts at 170°, and that of pyraconitine melts at 249°.

[1] Carr, *Trans.*, 1912, 101, 2241.
[2] Brady, *Trans.*, 1913, 103, 1821.
[3] Barger and Field (*Trans.*, 1915, 107, 231) have later established the identity of these substances and suggested other formulæ.
[4] Brady, *Trans.*, 1913, 103, 1821.
[5] *Arch. Pharm.*, 1913, 251, 453.

The Alkaloids of Aconitum Vulparia (*A. Lycoctonum* of authors).—The previous work on these alkaloids has been considerably added to and modified by Schulze and Bierling:[1]

Lycaconitine.—The formula of this alkaloid is now to be regarded as $C_{36}H_{46}O_{10}N_2$. It is a weak base and has $[\alpha]_D$ in alcohol $+42.5°$. No crystalline salt could be prepared. By acid hydrolysis it yields succinic acid and anthranoyl-lycoctonine. Alkaline hydrolysis produces lycoctonine and lycoctonic acid.

Myoctonine, according to the new work, has the formula $(C_{36}H_{46}O_{10}N_2)_2$. It will not crystallise, and no crystalline derivatives could be prepared. It has $[\alpha]_D + 44.8°$ in alcohol. This alkaloid yields lycoctonine and lycoctonic acid on hydrolysis by acids or alkalis.

Lycoctonine $C_{25}H_{39}O_7N,H_2O$, has different properties from those previously ascribed to the hydrolytic alkaloid which has also been named Lyaconine. It is well crystallised and colourless; it melts at $131°-133°$, and has $[\alpha]_D + 49.6°$ in alcohol. It contains four methoxyl-, a methylimino- and two hydroxy groups. It is a strong base, and forms crystalline salts. The *hydrochloride*, $(B.HCl,H_2O)$, forms prisms, m. p. $75°$; the *hydrobromide*, $(B.HBr,2H_2O)$, melts at $88°-89°$.

Lycoctonic acid $C_{11}H_{11}NO_5$, appears to be succinanilcarboxylic acid

$$C_6H_4{\begin{array}{l}\diagup COOH\\\diagdown NH.CO.CH_2.CH_2COOH\end{array}}$$

Anthranoyl-lycoctonine, $C_{32}H_{44}O_8N_2$, which results from the acid hydrolysis of lycaconitine, melts at $154°-155°$. It forms brown leaflets readily. soluble in chloroform, but less so in other solvents. It yields lycoctonine and anthranilic acid on alkaline hydrolysis.

All the alkaloids of this group exert powerful toxic effects on the heart; 0.01 grm. of lycaconitine and of myoctonine suffice to stop the frog's heart in 7 hours.

Assay of Aconite and its Preparations.—In the British Pharmacopœia, 1914, an alkaloidal standard for aconite root and its preparations is introduced. The following method of assay is directed for the root:

Into a small stoppered glass percolator, provided with a glass tap and suitably plugged with cotton wool, introduce 10 grm. of aconite root in No. 40 powder and 75 c.c. of alcohol (70%). Macerate for 4 hours, shaking occasionally. Then allow percolation to proceed slowly until the liquid ceases to drop. Continue the percolation by the addition of more of the same menstruum until 150 c.c. have been collected or the root is exhausted. Evaporate the percolate to dryness in a shallow porcelain evaporating basin, at a temperature not exceeding $60°$. Dissolve the residue in 5 c.c. of $N/10$ solution of sulphuric acid diluted with 20 c.c. of water. Filter into a separating funnel, washing the dish, and filter with about 30 c.c. of water. Add

[1] *Arch. Pharm.*, 1913, 251, 8.

to the mixed filtrate and washings 25 c.c. of ether and 2 c.c. of solution of
ammonia, and shake for 1 minute. After separation draw off the lower
layer into a flask, and filter the ethereal solution into a beaker. Return
the contents of the flask to the separator, add 20 c.c. of ether and again
shake for 1 minute, separating the aqueous liquid and filtering the ethereal
solution into the beaker. Repeat the operation with two other portions,
each of 20 c.c. of ether. Evaporate the mixed ethereal solutions to dryness,
dry the residue at 60°, dissolve it in 5 c.c. of $N/20$ solution of sulphuric
acid diluted with 20 c.c. of water, and titrate back with $N/20$ solution of
sodium hydroxide, tincture of cochineal being used as indicator. Deduct
the number of c.c. of the alkaline solution required from 5, multiply the
difference by 0.3217; the result will be the percentage of ether-soluble
alkaloids in the powdered root.

The Pharmacopœia requires that the root shall contain not less than 0.4%
of alkaloid when assayed by the above process and that the tincture shall be
standardised to contain 0.04%.

Attention[1] has been drawn to the importance attaching to the comparison
of weight and titration values; the theoretical figure 0.06434 grm.
per c.c. of $N/10$ acid is frequently exceeded, showing that other bases are
present. It is to be observed that it is important to extract the acid aqueous
liquor with ether very completely before rendering alkaline to extract the
alkaloid and that the acidity of this liquor should not, at the same time, be
excessive.

Toxicological Detection of Aconite.—Fühner[2] points out that in forensic
cases aconitine may best be detected by observing its action upon the isolated
frog's heart. The peristalsis shown on the tracings is characteristic. 0.005
mg. may be detected in this way. While the tingling on the tongue is a
valuable indication, a similar effect, though weaker, is produced by veratrine.

[1] Evan's Analytical Notes, 1911, 12 and 13.
[2] Arch. Exp. Path. Pharm., 1911, 66, 178.

ATROPINE AND ITS ALLIES.

By FRANCIS H. CARR.

TROPEINES AND SCOPOLEINES.

Their constitution being well known, the chemistry of atropine and hyoscyamine has undergone little advance since the publication of Vol. VI. The new derivatives which have been described, such as the sulphuric acid esters[1] and the methyl methosulphites[2] do not, from their character, call for further description in the present work.

The alkaloid pseudo-hyoscyamine which was previously regarded as an isomer of atropine and hyoscyamine, has been shown[3] to differ in composition from these alkaloids. It is devoid of the methyl group attached to the nitrogen atom, having the formula $C_{16}H_{21}O_3N$ and it has consequently been renamed *norhyoscyamine*. Its racemic modification noratropine has also been prepared by Carr and Reynolds. When hydrolysed with alkalis, these alkaloids yield nortropanol and tropic acid. They may be represented by the formula

$$CH_2\text{-----}CH\text{-----}CH_2 \qquad C_6H_5$$
$$\qquad\qquad | \qquad\qquad |$$
$$\qquad NH \qquad CH.O.CO.CH.$$
$$\qquad\qquad | \qquad\qquad |$$
$$CH_2\text{-----}CH\text{-----}CH_2 \qquad CH_2-OH$$
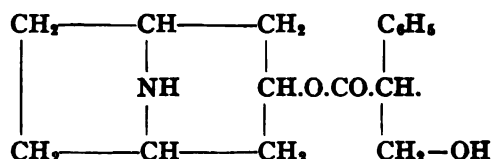
Noratropine and norhyoscyamine react readily with methyl iodide, forming atropine and hyoscyamine respectively, and by using other alkyl iodides homologous N-alkyl derivatives are formed.

Hyoscine.—The constitution of hyoscine (scopolamine) remains unknown, but the work of Willstätter and his collaborators[4] indicates that one of the oxygen atoms of scopoline (the base derived by hydrolysis) is attached by an ether-like linking, the other being present as an hydroxyl. Further, Tutin[5] has shown that scopoline may be obtained in its optically active form, and must contain either one or two asymmetric carbon atoms; if the latter be the case, the asymmetric groupings must be similar.

The following new substances in this group of alkaloids call for further description.

[1] Hoffman La Roche & Co., D.R.P., 247455 and 247457.
[2] Gerber, D.R.P., 228204.
[3] Carr and Reynolds, *Trans.*, 1912, 101, 974.
[4] *Zeit. physiol. Chem.*, 1912, 79, 146.
[5] *Trans.*, 1910, 97, 1793.

Noratropine, $C_{16}H_{21}O_2N$, is a white crystalline base, m. p. 113°–114°; it readily combines with water, forming a monohydrate, m. p. 73°. Noratropine and its salts are optically inactive.

Noratropine hydrochloride, (B,HCl), crystallises from a mixture of alcohol and acetone in silky needles m. p. 193°; *noratropine sulphate,* (B₂,H₂SO₄), separates from water in long needles, m. p. 257°; *noratropine aurichloride,* (B,HAuCl₄), forms rosettes of leaflets which melt under hot water, and crystallise on cooling, m. p. 157°.

Norhyoscyamine, $C_{16}H_{21}O_3N$, has been shown by Carr and Reynolds[1] to occur in *Datura metel* 0.01%, *Datura meteloides* 0.02%, *Duboisia myoporoides* 0.15% and *Scopolia japolica* 0.03%. These authors also point out that there is evidence to show that it occurs in *Datura fastuosa* and *Mandragora vernalis.* Norhyoscyamine crystallises in prismatic needles, m. p. 145.5°. The specific rotatory power of the pure base in 50% alcohol is $[\alpha_D] - 23.0$.

Norhyoscyamine hydrochloride, (B,HCl), forms rosettes of needles from alcohol and ether, m. p. 207°, norhyoscyamine *sulphate,* (B₂,H₂SO₄, 3H₂O), crystallises from acetone and water in long slender silky needles, m. p. 249°; norhyoscyamine *aurichloride,* (B,HAuCl₄), separates from alcohol and water in glistening golden yellow scales, m. p. 178°–179°.

Detection and Estimation of Tropeines.—It has been pointed out that the properties of the gold salts and picrates of these alkaloids constitute the best means of identifying them when sufficient substance is available. Carr[2] shows that when working with pure alkaloid ¹⁄₁₀ grain may be identified by the aurichloride and ¹⁄₅₀ grain by the picrate method.

The following table gives the melting point of the chief salts of the alkaloids of this group.

	Hyos-cyamine	Atropine	Norhyo-scyamine	Nor-atropine	l-Hyo-scine	i-Scopol-amine
Alkaloid.............	107°	116–117°	140.5°	113–114°	....	....
Hydrochloride........	....	163	207	193	200°	....
Sulphate.............	205–209	194	249	257	....	....
Oxalate..............	176	196–197	245	247–248	....	....
Aurichloride..........	165	137–139	178–179	157	198–200	208°
Picrate..............	165–166	175–176	220	227	180–181	193

The test for atropine and hyoscyamine described in paragraph *g*, on page 307 of Vol. VI has been modified by Labat.[3] A particle of the alkaloid is placed in a test-tube and 2 c.c. of 10% sulphuric acid and 1 drop of a saturated aqueous solution of potassium chromate added. On warming, the characteristic hawthorn odour develops and changes to the odour of benzaldehyde. The latter odour is also produced when the alkaloid is boiled with a dilute solution of sodium hypobromite.

[1] *Loc. cit.*
[2] *Chem. World,* 1, 3.
[3] *Bull. Soc. Pharm. Bord.,* April, 1914, 148

For the assay of belladonna leaves the British Pharmacopœia 1914 gives the following method:

Into a small stoppered glass percolator provided with a glass tap and suitably plugged with cotton wool, introduce 10 grm. of belladonna leaves in No. 60 powder, and 50 c.c. of a mixture of chloroform 1 volume and ether 4 volumes. Shake, set aside for 10 minutes, then add 2 c.c of solution of ammonia diluted with 3 c.c. of water, and set aside for 1 hour, shaking frequently. Then allow percolation to proceed slowly, receiving the percolate in a separator containing 6 c.c. of $N/1$ solution of sulphuric acid diluted with 20 c.c. of water. When the liquid ceases to pass, continue the percolation with a further 50 c.c. or more of the ether-chloroform mixture, added in small quantities until the leaves are exhausted. Shake the separator well, and, after separation, draw off the acid liquid into a second separator. Repeat the extraction of the ether-chloroform solution with two successive portions, each of 10 c.c. of the diluted acid. Make the mixed acid solution alkaline with solution of ammonia, and shake out with three successive portions of 15, 15, and 5 c.c. of chloroform. Evaporate the mixed chloroform solutions to dryness, dissolve the residue in 3 c.c. of ether, and again evaporate to dryness. Dissolve the residue in 10 c.c. of $N/20$ solution of sulphuric acid and titrate with $N/20$ solution of sodium hydroxide, tincture of cochineal being used as an indicator. Deduct the number of c.c. of the alkaline solution required from 10, multiply the difference by 0.1446; the product will be the percentage of alkaloids in the leaves.

The method given for the assay of liquid extract of belladonna in the 1914 B. P. is similar to that given in the previous B. P. but omits the first alkaline extraction. The following method is given for the assay of dry extract of belladonna, tincture of belladonna and extract of hyoscyamus:

Evaporate 100 c.c. in an evaporating basin on a water-bath, until it measures about 10 c.c., add, if necessary, sufficient alcohol (90%) to dissolve any separated substance, and transfer to a separator, rinsing the dish with a little water. Add 10 c.c. of water, 20 c.c. of chloroform, and 2 c.c. of solution of ammonia. Shake well and separate the chloroform layer. Repeat the extraction with two successive portions of 10 c.c. of chloroform. Mix the chloroform solutions, and shake them with 10 c.c. of $N/1$ solution of sulphuric acid, diluted with twice its volume in water. Separate the chloroform solution. Repeat the shaking with a further 10 c.c. of the acidified water. Mix the acid solutions, add 20 c.c. of chloroform and 4 c.c. of solution of ammonia. Shake well, draw off the chloroform solution into a beaker, and repeat the extraction with two further portions, each of 10 c.c. of chloroform. Allow the mixed chloroform solutions to evaporate, dry the residue on a water-bath 30 minutes, dissolve it in 10 c.c. of $N/20$ solution of sulphuric acid and titrate with $N/20$ solution of sodium hydroxide, using tincture of cochineal as indicator. Deduct the number of c.c. of alkaline solution required from 10, and multiply the difference by 0.01446; the prod-

uct will be the weight in grams of the alkaloids contained in 100 c.c. of the tincture.

The B. P., 1914, adopts the following standards:

Belladonna leaves	Not less than 0.3 % of alkaloid.
Belladonna root	No standard.
Belladonna plaster	0.25 % of alkaloid.
Belladonna ointment	0.6% of alkaloid.
Ext. of belladonna dried	0.95 to 1.05 % of alkaloid.
Ext. of belladonna liquid	0.75 % w/v of alkaloid.
Ext. of hyoscyamus	0.3 % of alkaloid.
Tinct. of belladonna	0.035 % w/v of alkaloid.

A suggestion[1] has been made that for the determination of atropine the insoluble silicotungstate should be precipitated and weighed. The salt is soluble less than 1 part in 40,000 and has the composition $12WO_3$, SiO_2, $2H_2O,4B$, after drying at 20°.

ERRATA IN VOL. VI.

Page 256, last line, for "*acetyl*" read " *acyl*."

Page 297, last line, for "page 291" read pages 291 and 303."

Page 298, line 6 from bottom, for "Atropamine.—Apo-atropine" read "Atropamine-Apo-atropine."

Page 299, line 3 from bottom, "page 291" should read "pages 289 and 290."

[1] O. Javillier, *Bull. Sci. Pharm.*, 1910, 315

COCAINE.

By SAMUEL P. SADTLER.

Separation and Identification of Cocaine.—H. C. Fuller[1] gives the following method:

Solid substances are dissolved in water or in $N/1$ sulphuric acid, or if necessary they are extracted with alcohol containing ammonia, water is added, and the bulk of the alcohol evaporated. Syrups should be diluted to the consistence of a 50% sugar solution and freed from gum and fat when present. The solution, containing a slight excess of ammonia, is shaken in a separating funnel with three successive portions of 50 c.c. of Prolius mixture (ether 4 parts, chloroform 1 part, alcohol 1 part), and the combined extracts are filtered and evaporated nearly to dryness on a hot water-bath. The moist residue is taken up with 25 c.c. of $N/1$ sulphuric acid, added in portions of not more than 10 c.c., the mixture being warmed and filtered after each addition, and is finally washed with a little water. The acid solution is shaken with five successive portions (15 c.c. each) of chloroform, and the united chloroform extracts washed with 10 c.c. of water, which is added to the acid solution, whilst the chloroform is discarded. The acid solution is next shaken with 10 c.c. of petroleum benzin (b. p. 40–60°), the extract discarded, a slight excess of ammonia added to the acid solution, and the extraction thrice repeated with 15 c.c. of benzin each time. The united benzin extracts are washed once with water, filtered, and evaporated rapidly on the water-bath in a current of air. The residue will then contain any cocaine that was originally present. A test is made for alkaloids in a portion of the residue with mercuric iodide solution, and if a precipitate is obtained the remainder of the residue is heated with 2 c.c. of strong nitric acid on the water-bath until there is no further odour of nitric acid, and then cooled and treated with 5 to 10 drops of $N/5$ alcoholic potassium hydroxide solution. On gently warming the dish, cocaine, when present even in minute traces, gives off the odour of ethyl benzoate. A purple colouration indicates the presence of atropine, strychnine or yohimbine, though in some instances a similar colouration is given by the residue from the coca leaf. An odour of ethyl benzoate is also produced by tropacocaine, benzoylecgonine and aconitine, but the first two may be differentiated by means of the microscope, whilst benzoylecgonine and aconitine are not removed to any great extent from the aqueous solution by benzin. Cocaine

[1] *Techn. Division of Drugs, U. S. Dept. Agric., Bureau of Chem.*, Bull. 150, April 22, 1912, 41-43.

may also be identified by the crystalline compound which it gives with gold chloride. Moreover, when heated for $1\frac{1}{2}$ hours in a strong closed flask on the water-bath with 15 c.c. of dilute hydrochloric acid and a few crystals of salicylic acid, cocaine will give rise to an odour of oil of wintergreen, whereas tropacocaine does not give this reaction. This test might also indicate cinnamylcocaine and the truxillins, but, on the other hand, none of these gives the ethyl benzoate reaction.

Differentiation of Cocaine from Its Substitutes.—D. Sherbatshev[1] describes the following method. Three reagents are required: (1) 10% aqueous solution of ammonia; (2) aqueous potassium hydroxide (1:10); (3) saturated aqueous sodium hydrogen carbonate. Three drops of the solution under examination are placed separately on an object glass by means of a glass rod. To one of these drops is added a drop of solution No. 1, to the second a drop of No. 2, to the third a drop of No. 3. A precipitate may or may not be formed in one or all cases. A transitory precipitate (*i.e.*, one sol. in excess of reagent) is not to be considered a precipitate. In the event of the presence of any of the following compounds, the following are the indications:

	NH₄OH	KOH	NaHCO₃
Stovaine	Ppt.	Ppt.	Ppt.
β-Eucaine		Faint ppt.	
Nirvanine	Ppt.	Ppt. dissolves.	Ppt.
Alypine	Ppt.	Ppt.	
Holocaine	Ppt.	Ppt.	Ppt.
Novocaine		Ppt.	

Stovaine and holocaine yield precipitates with each reagent. However, since holocaine is little used on account of its toxicity, a precipitate with each reagent is strongly indicative of stovaine. The behaviour of β-eucaine and novocaine is likely to mislead, since the faint precipitate yielded by the former is not sufficiently characteristic.

[1] *Apoth. Ztg.*, 27, 441.

OPIUM ALKALOIDS

By FRANK O. TAYLOR.

The short time that has elapsed since the publication of Vol. VI renders any extensive revision of the chapter on Opium Alkaloids unnecessary, although there has been no diminution of work on analytical processes applicable to opium and its alkaloids during the past 3 years, as compared with preceding years. Quite a little new work has been published, but no very radical changes of former methods are involved and the exact estimation of morphine either in opium or in medicinal compounds remains one of the most difficult of all the alkaloidal assays, and the most fruitful in provoking discussion and criticism. The writer will endeavour to give sufficient information regarding new tests or modifications of old methods to permit of their satisfactory use, but detailed discussion cannot be attempted in most instances, and it seems unnecessary to refer to any published papers which do not present any really new material. For convenience of reference the subheads of this chapter will be identical with those in Vol. VI, and the page numbers will frequently be referred to as an added help in connecting this new material with the old.

Constitution of Opium Bases (Vol. VI, 355-361).—Some additional work has been done on the constitution of the opium alkaloids and their derivatives, but as this does not necessitate any pronounced change in statements already made and has no bearing on analytical processes there is no need to embody certain of these theoretical considerations in this chapter.

Behaviour of Opium Bases with Solvents (Vol. VI, 362).—Some older work not referred to in Vol. VI is that of Schindelmeiser[1] on the solvent action of carbon tetrachloride on alkaloids, among which the following determinations should be recorded here. The figures are grm. dissolved in 100 c.c. at 17°: Morphine 0.032 grm., codeine 1.328 grm., papaverine 0.203 grm., narceine 0.011 grm.

The solubility of morphine and narcotine in acetone, water and mixtures of these has been investigated by Guerin[2] who used these solubilities as the basis of a modified opium assay (see page 505).

Figures indicate amount dissolved in 1000 c.c. at 15°.

[1] *Chem. Zeit.*, 1901, 25, 129.
[2] *J. Pharm. Chim.*, 1913, 7, 438.

	Anhydrous acetone	Acetone and water, equal parts	Water
Morphine.....................	1.28 grm.	1.32 grm.	0.288 grm.
Narcotine....................	41.96 grm.	0.70 grm.	0.1 grm.

For new determinations on dionin see that heading.

Colour Reactions of Opium Bases (Vol. VI, 366–370).—For several new colour reactions, some of which are quite useful, see under the proper alkaloidal headings.

Salts of Morphine (Vol. VI, 376–379).—*Morphine sulphate* as obtained commercially has been found frequently to contain considerable amounts of codeine sulphate as an impurity. Engelhardt and Jones[1] in four samples found from 1.45% to 3.97%, and Williams[2] in five samples found from 0.9% to 7.0%, while in tablets he found codeine sulphate to the extent of 2.5% to 6.5% of the morphine sulphate present. He demonstrated that morphine when precipitated from even a dilute solution containing codeine was liable to carry a part of the codeine along with it, thus accounting for its presence in commercial morphine salts. For method of estimation see under the sections on Codeine.

Engelhardt and Winters[3] compare several methods of estimating the purity of morphine salts and find that morphine nitrate rarely runs above 90% of the theoretical purity; they do not indicate what the impurities are or whether this is solely a case of a different amount of water of crystallisation than is ordinarily calculated. They find morphine acetate also contains somewhat below 100% of the theoretical strength.

Detection and Estimation of Morphine (Vol. VI, 379–387).—This section as in Vol. VI deals chiefly with qualitative tests, though some of them are capable of elaboration for roughly quantitative work.

Fabinyi[4] reports on a colour test for morphine originally devised by Radulescu as follows: To the solution to be tested add a few drops of dilute hydrochloric or sulphuric acid, then a very small quantity of sodium nitrite either in dry form or solution, and finally make alkaline with either ammonia or sodium or potassium hydroxide. In the presence of morphine a red colour at once appears which is destroyed by acid and restored by alkali. When the solution is very dilute the colour is more of a mahogany shade. In strong solutions a green colour is produced on adding the sodium nitrite and before the alkali is added. The same test is reported on by Radulescu[5] who tried it on 150 substances and found but one (a lettuce extract of doubtful purity) that gave a similar indication. He considers that the colour probably depends on the formation of nitroxanthranol. This colour is not extracted by chloroform, carbon disulphide or ether. The test has the great advantage of

[1] *Drug. Circ.*, 1911 555.
[2] *Am. J. Pharm.*, 1912, 84, 391.
[3] *J. Am. Pharm. Assoc.*, 1915, 4, 288.
[4] *Oesterr. Chem. Zeit.*, 1912, 15, 61.
[5] *Boll. chim. farm.*, 1913, 51, 865.

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being applicable to many compounds without extracting the morphine in approximately pure form.   The writer has had so far only a limited experience with the test but has verified its usefulness in showing the presence of morphine directly in solutions of compound tablets and in mixtures such as Tr. opium camphorated.   It accurately distinguishes morphine from codeine and dionin, which give no colour with the test, but is not so dependable in the case of heroin as this substance gives a slight colour almost identical with that of morphine in very dilute solution, so by this test alone one cannot be certain whether there is present only a minute quantity of morphine or a large amount of heroin.   With equal amounts of the two the colour from the morphine is very much more intense.   This test also distinguishes morphine from apomorphine, which alkaloid gives a peculiar and characteristic reaction described under apomorphine (page 499).

Aloy and Rabaut[1] report further experiments on the test with uranium acetate or nitrate (see Vol. VI, 386).   It is preferable to add a small crystal of uranium nitrate to the solutions under test rather than a 5% solution.   They now claim it will detect as little as 0.05 mg. of morphine, but to do so the conditions must be the best possible and it is not so delicate as a number of other tests; furthermore it is not characteristic of morphine but is given by many substances which contain a phenolic hydroxyl group.

Oliver has very recently[2] described as a new test one based on the catalytic action of copper, apparently being unaware that Denigès in 1910 published what is practically the same test (see Vol. VI, 386).   However, his peculiar method of applying it seems to render it more delicate than in the form originally proposed by Denigès, or at least this is indicated by the writer's brief experience with the modified test.   To the solution under test add 1 or 2 c.c. of hydrogen peroxide solution and sufficient 28% ammonia to make distinctly alkaline, and stir with a bright copper wire when a port wine or dark cherry colour quickly develops.   The use of the wire enables a more delicate adjustment of the test than by the use of copper sulphate solution.   To render it more delicate add a little solution of potassium cyanide after stirring with the copper wire, to destroy any blue colour from the copper and ammonia.   The test will detect in a pure solution as little as 0.02 mg. of morphine.   The writer has verified the statement of Denigès, which is contrary to that of Oliver, that heroin gives a similar indication; in fact this alkaloid responds to the test even more quickly than morphine and the colour is almost identical.   Codeine and dionin give no colour, but apomorphine gives a raspberry red that fades rather quickly.

The peculiar effect of morphine on white mice is made the basis of a biological test by Hermann.[3]   These animals treated hypodermically with morphine become abnormally active and excitable, especially to noises, and the tail assumes an S-shaped curve over the back.   It is stated that 5 mg. gives

[1] *Bull. Soc. Chim.*, 1914, 15, 680.
[2] *Med. Chronicle*, 1914, 27, 221.
[3] *Biochem. Zeit.*, 1912, 39, 216.

a reaction in 2 minutes which persists over 20 hours, and 0.01 mg. showed an effect in 12 minutes which lasted 1-2 hours. This test is not specially useful but can be employed to check preliminary solutions obtained in forensic work.

Apomorphine (Vol. VI, 387-389).—In the course of certain experiments V. Paolini[1] found that the hydrochloride contained an average amount of 4.2% of water or 0.75 H_2O in each molecule. He also prepared a *dibenzoate*, m. p. 156°, by the action of benzoyl chloride in presence of pyridine.

The formation of apomorphine in morphine solutions (see Vol. VI, 388) has been carefully investigated by Feinberg[2] who finds it is not produced by long-continued boiling of solutions of morphine, its hydrochloride or other salts, nor by long standing either with or without the presence of nutrient media to promote bacterial action.

Feinberg also describes a test for apomorphine in presence of morphine. To a solution add 3 drops of 1% solution of potassium ferricyanide and shake with 1 c.c. of benzene. In presence of apomorphine the benzene is coloured amethyst-violet, and on adding a few drops of sodium hydroxide solution and shaking, the colour changes to violet-red and, on long standing, to violet. This is a very delicate test, but it is not at all necessary to use the ferricyanide, as the addition of a few drops of ammonia and shaking with benzene gives the same results, only a little more slowly.

On making a solution of apomorphine slightly acid with hydrochloric or sulphuric acid and then adding a very little sodium nitrite, a magnificent deep cherry-red colour appears. The colour is best shown in very dilute solution and with small quantities of reagents. The test serves not only to distinguish apomorphine from morphine, but will detect a very small amount of the former in the presence of much morphine. No colour is imparted to ether or chloroform on shaking with them, but on adding ammonia the colour changes to a dirty green, and then on shaking with ether or chloroform these solvents assume the characteristic violet colour derived from apomorphine as in the preceding test.

The test given in Vol. VI, p. 389, for β-chloromorphide in apomorphine should specify a solution of sodium bicarbonate instead of the carbonate.

Heroin (Vol. VI, 389).—The colour test with hexamethylene-tetramine and sulphuric acid is best applied by using as a reagent 10 c.c. of the concentrated acid mixed with about 0.5 c.c. of 10% solution of hexamethylene-tetramine. To a few c.c. of this, add either a crystal of heroin or its salts or a very little solution, when a fine purplish or violet colour at once appears. This is not characteristic, as morphine and codeine give similar colours as do other substances of a phenolic character. The test is practically identical with that given by formaldehyde and sulphuric acid.

In the absence of morphine or other interfering substances Miller[3] uses

[1] *Atti. accad. Lincei*, 1913, 22, II, 121; *Chem. Abst.*, 1914, 79.
[2] *Zeit. physiol. Chem.*, 1913, 84, 363.
[3] *Am. J. Pharm.*, 1915, 87, 248.

the formaldehyde-sulphuric acid reaction for the colourimetric estimation of heroin and reports that it is applicable in the case of mixtures of cocaine and heroin by determining the total amount of alkaloids by weight, then estimating the morphine by this colour reaction and deducting the quantity so determined from the total. The method is, of course, not strictly accurate. For details see the original paper.

Harris and Clover[1] find that commercial heroin hydrochloride and diacetylmorphine hydrochloride contain about 5% of water of crystallisation, which is easily removed at 100° but is quickly reabsorbed, especially in moist air. If diacetylmorphine hydrochloride is prepared from the anhydrous alkaloid by precipitation from a benzene solution with hydrochloric acid, an anhydrous salt is obtained which is not hygroscopic.

Dionin (Vol. VI, 390).—The melting point is not at all sharp and more recent work indicates that the figures given in Vol. VI should be revised. Schaefer[2] and Dott[3] agree that the melting point of the base (ethyl-morphine) is about 110° to 115°, though it begins to soften at 88° if not well dried before taking the melting point. On drying the hydrochloride at 120°-125° Dott found that it did not melt till about 170°, but this is rather the melting point of its decomposition products which have begun to form at this temperature.

Schaefer (*loc. cit.*) records its solubility as follows:

	Water		Alcohol		Ether
	15°	25°	15°	25°	25°
Ethylmorphine.....................	....	1 : 480	....	1 : 15	1 : 75
Ethylmorphine hydrochloride...........	1 : 11.5	1 : 8	1 : 26	1 : 20	....

He also suggests a modification of Hesse's test, to indicate purity and distinguish it from codeine. To 2 c.c. of solution containing 0.05 grm. of the hydrochloride add 3 drops of 10% ammonia. When pure, the solution remains clear and on standing deposits crystals of alkaloid. When impure, the solution becomes milky and the formation of crystals is greatly retarded. A 1 : 100 solution of codeine hydrochloride remains clear, giving no precipitate or crystals.

Codeine (Vol. VI, 390-395).—In the manufacture of morphine, codeine is obtained from the mother liquors after precipitation of the morphine, but more or less of the codeine is frequently precipitated with the morphine even though the volume of water is theoretically sufficient to retain in solution several times the quantity of codeine present. The result, as noted on page 497, is the presence of codeine sulphate as an impurity in morphine sulphate. It may best be determined by Williams' method.[4]

[1] *J. Am. Pharm. Assoc.*, 1915, 4, 291.
[2] *Am. Jour. Pharm.*, 1912, 84, 389.
[3] *Pharm. J.*, 1913 (4), 36, 99.
[4] *Am. Jour. Pharm.*, 1912, 391.

Dissolve 0.5 to 1.0 grm. of the morphine salt, or tablets equivalent to this, in 15 to 20 c.c. of water, then add 5% solution of sodium hydroxide till the precipitate first formed is redissolved (3 or 4 c.c.). Shake out with four 20 c.c. portions of chloroform and the combined chloroform extracts with 10 c.c. of water slightly alkaline with sodium hydroxide, draw off the chloroform from the separator through a pledget of cotton wet with chloroform, wash the separator with two 10 c.c. portions of chloroform filtering through the same cotton. Evaporate the solution carefully to dryness, dissolve residue in 5 c.c. $N/10$ sulphuric acid and titrate the excess with $N/50$ alkali using cochineal as indicator. Each c.c. of $N/10$ acid equals 0.0315 grm. codeine alkaloid or 0.039 grm. sulphate. ·

In the examination of morphine sulphate a test for codeine should always be made and not more than 1% to 1.5% should be present in good morphine sulphate. A test to this effect is included in the U. S. P., 9th Revision, the test being practically identical with that described above and limiting the amount of codeine sulphate in morphine sulphate to not more than 1%. There is a test that serves this purpose in several of the European pharmacopœias at present.

Aporeine (Vol. VI, 396), $C_{18}H_{19}O_2N$.—Pavesi,[1] who first isolated this alkaloid, has done considerable additional work on it. At 88°–89° it melts to a fluorescent liquid which becomes brown in the air at about 225° but not at 280°–290° in hydrogen or carbon dioxide, in which it can be distilled. It is quite soluble in most organic solvents; in petroleum ether it gives an 11% solution at the boiling point and 3.5% at 15° to 20°. It forms a *hydrobromide* in yellowish pearly scales, which beomes discoloured at 190° and melts with decomposition at a temperature considerably above 210°. The *sulphate* forms filamentary crystals that are unstable in light and air; the *nitrate* is more stable. The *oxalate* forms white tablets, m. p. 89°–90°; the *citrate* forms needles, m. p. 81°–82°; the *tartrate* melts with decomposition at 190°. The *acetate*, *benzoate*, and *salicylate* are of a resinous character.

Narcotine (Vol. VI, 400–403) is not likely to occur as an impurity in morphine salts, but has recently been used in combinations with morphine for certain medicinal purposes under various names (see Morphine-narcotine Meconate, page 503). To detect narcotine in morphine salts, heat a little with concentrated sulphuric acid on the water-bath. In the absence of narcotine not more than a slight violet colour should appear. With narcotine this test gives a decided violet colour, but is not definite with less than 0.4 to 0.5% of narcotine on account of the colour given by morphine alone. A more sensitive test given by Labat[2] consists in mixing the morphine salt to be tested with 2 c.c. of sulphuric acid and 0.2 c.c. of alcoholic solution of tannic or gallic acid and heating on the water-bath, when a blue colour is given with much narcotine, or greenish if the latter is present in small amount.

[1] *Gazzetta*, 1914, 44, I, 398.
[2] Through *Yearbook Pharm.*, 1913, page 25.

In the spectroscope this solution gives an absorption band near the infra-red with a 1 : 1000 solution.

Thebaine (Vol. VI, 405–406).—In *Papaver orientale* Klee[1] has found both thebaine and a new alkaloid which he calls

Iso-thebaine, $C_{17}H_{14}N(OCH_3)_2OH$, m.p. 203°–204°; $[\alpha]_D = +285.1°$ in alcohol. It gives crystalline salts with sulphuric and hydrochloric acids and forms an *l*-bitartrate. On treating with nitric acid it gives an intense violet colour becoming brownish and then reddish-yellow, which is said to be characteristic. It may be separated from thebaine by heating with dilute hydrochloric acid which converts the thebaine into thebenine, insoluble in ether, but does not affect the *iso*-thebaine.

There has recently been a tendency to use for medicinal purposes a mixture of some of the opium alkaloids or their salts in a pure form, owing to the fact, now fairly well established, that morphine alone differs in its physiological action from a mixture with other alkaloids, particularly nar-cotine, and the analysis of these may be mentioned here.

Pantopon, stated to be the hydrochlorides of the combined opium alka-loids, has been examined by Mannich and Schwedes[2] with the following result:

Morphine	47.5%	H₂O	9.5%
Narcotine	11.2%	HCl	9.4%
Codeine	6.4%	Mineral substances	0.3%
Other opium bases	10.9%		

Opiopon is a similar preparation, but Mannich and Schwedes found that the composition of two different samples varied greatly.

Opon is like the two preceding products except that it contains no morphine.

The estimation of each alkaloid separately, except a few of the most im-portant, is out of the question as it is a matter of extreme difficulty, but for practical purposes the estimation of morphine, narcotine and codeine is sufficient and in some cases that of morphine alone.

Anneler[3] reports on the investigation of several methods of estimating morphine and prefers, of the crystallisation methods tried, that of the British Pharmacopœia (Vol. VI, 423) as modified by Debourdeaux (see page 507). Of the shake-out methods the following is the one that gave the best results which, however, were about 1.5% too high:

Dissolve 0.8 to 1.0 grm. in 30 c.c. of water and add a concentrated aqueous solution containing 1.0 grm. of sodium hydrogen carbonate. Shake out with three 10 c.c. portions of chloroform saturated with morphine, agitating gently to avoid an emulsion. The chloroform solution is drawn off and filtered through paper wetted by chloroform. 60 c.c. of a mixture of equal parts of chloroform and *iso*butyl alcohol are then passed through this same filter into the separator containing the original aqueous solution. Shake for 10 minutes

[1] *Arch. Pharm.*, 1914, 252, 211.
[2] *Apoth. Zeit.*, 1913, 28, 82.
[3] *Arch. Pharm.*, 1912, 250, 186.

and draw off into another separating funnel. Repeat the extraction with 20 and 10 c.c. of the same mixture. The united extracts are then shaken with 10 c.c. of water, the water discarded, the chloroform-*iso*butyl alcohol solution filtered into a clean separator through a filter wet with chloroform. Shake out with 20 c.c. of $N/10$ hydrochloric acid and wash with two 10 c.c. portions of water. Titrate the combined acid solution with $N/10$ alkali, using iodeosin indicator in the presence of 30 c.c. of ether.

Morphine-narcotine Meconate, known also under the name **Narcophine,** is a double salt of morphine, containing equi-molecular quantities of morphine, narcotine and meconic acid, with 4 molecules of water of crystallisation. This theoretical constitution is not exactly carried out in practice, as is shown by comparison of the theoretical values and an analysis by Dott[1] shown in the table below:

	Theory	Analysis
Morphine	29.38%	31.3%
Narcotine	42.57%	38.6%
Meconic acid	20.61%	24.6%
Water	7.42%	5.5%

This difference is probably due to the feebly basic character of narcotine and to the tendency of meconic acid to behave in some respects like a tribasic acid. Narcophine obtained by interaction of the proper proportions of its three constituents in alcohol and precipitation by ether is completely soluble in 12 parts of water at 20°, which solution can be sterilised in the usual way. A physical mixture of the three does not give a clear solution in water. In this connection it is interesting to note that a mixture of ¼ grain (0.016 grm.) of morphine hydrochloride and the same of narcotine hydrochloride gives a clear solution in 1 c.c. of water that soon throws down a crystalline precipitate of narcotine, especially if heated.

An approximate assay of morphine-narcotine meconate may be made according to Dott (*loc. cit.*) by titrating the morphine directly with $N/10$ acid using cochineal as indicator. The end point is not distinct but fairly good results are obtainable, even in the presence of the narcotine and meconic acid. In similar manner the meconic acid may be titrated with $N/10$ alkali using phenolphthaleïn as indicator. From these results the narcotine and water may be calculated.

A more accurate but much longer method is to extract the narcotine by shaking the aqueous solution, made alkaline with ammonia, with four or five large portions of ether, evaporate the ethereal solutions to dryness and weigh or titrate. The morphine is estimated by collecting the crystalline precipitate, washing with alcohol saturated with morphine, drying and weighing or by redissolving the crystals with acid, making alkaline with ammonia and extracting with equal parts of chloroform and *iso*butyl alcohol, and afterwards drying and weighing. By a process of this kind, collecting the crystallised

[1] *Pharm. J.,* 1913 (4), 36, 99.

morphine as first mentioned, Puckner[1] obtained from narcophine, morphine 31.12% and narcotine 44.25% and found, by drying, 7.14% water.

The meconic acid may also be estimated by the colourimetric method described on page 506.

Opium.
(Vol. VI, 407).

Alkaloids (Vol. VI, 408).—Work by Van Itallie and Kerbosch[2] has shown that opium from Asia Minor, Persia, Egypt, China, France and America contained morphine, codeine, narcotine, narceine, thebaine and papaverine, but Indian opium from Bengal, Benares and the so-called Patna opium did not contain papaverine.

Mossler[3] gives results of an extended investigation of the amount of morphine in the capsules of the opium poppy under a number of conditions. The whole air-dried capsules contained 0.1369% of morphine, and those sterilised for half an hour in hot alcohol vapour to destroy ferments contained 0.130%. After the exudations of the latex from the scarified capsules a notable quantity of morphine remains in the capsules. In the ripe capsules the morphine falls to 0.053%.

Adulteration of Opium(Vol. VI, 417).—von Friedrichs[4] has investigated the effect of various moulds on opium alkaloids and reports that *Penicillium glaucum* and *Citromyces glaber* do not attack any of the important opium alkaloids. *Aspergillus niger* attacks narcotine and codeine but not morphine, whilst *Aspergillus ostianus*, found especially on Turkish opium, appears to decompose morphine slightly as well as narcotine and codeine.

Estimation of Morphine in Opium (Vol. VI, 417-433).—Several investigators have recently called attention to the fact that a portion of the morphine in opium is often in such a condition that it is insoluble in water; so that any assay process that involves preliminary extraction of the opium with water is liable to give low results. (See Debourdeaux,[5] Collard,[6] La Tour and Nalpasse,[7] and Shreve.[8]) Results obtained by Debourdeaux also indicate that in a powdered opium the amount of insoluble morphine increases with age but not at a definite rate, and at the same time the total amount of morphine decreases slightly, probably due to the action of an oxydase. Latour and Nalpasse (*loc. cit.*) state that even dilute alcohol fails to completely extract morphine from opium in all cases, so that Sydenham's laudanum of proper strength cannot be made from an opium of exactly 10% strength. In this same preparation Debourdeaux reported a marked loss of morphine as shown

[1] *Rep. Lab. Am. Med. Assoc.*, 6, 92.
[2] *Arch. Pharm.*, 1911, 248, 609.
[3] *Pharm. Zeit.*, 1914, 59, 600.
[4] *Zeit. physiol. Chem.*, 1914, 93, 276.
[5] *J. pharm. chim.*, 1911, 4, 13; 1912, 6, 491 and 542.
[6] *Yearbook Pharm.*, 1913, 359.
[7] *Ann. Fals.*, 6, 289.
[8] *J. Ind. Eng. Chem.*, 1912, 4, 514.

by assay, with ageing, especially when exposed to the air. A specimen assaying 1.024% morphine in 1908 fell to 0.851 in 1912 and another dropped from 1.071% to 0.883 in the same time. While a precipitate was formed, this contained no perceptible traces of morphine. These results, so far as the writer knows, have not been verified by other workers, and it seems rather extraordinary that so stable an alkaloid as morphine should be decomposed in a preparation such as this while fluid extracts of drugs like ipecac and physostigma show practically no loss of alkaloid in the same length of time.

As far as general methods of estimating morphine are concerned, nothing better than one of the various modifications of the lime process has been devised. That of the B. P., 1898, as modified by Dowzard (Vol. VI, 423 and 425) is excellent (see also Jensen[1]).

The B. P. 1914 assay is practically identical with that of the 1898 edition except that the crystals are dried at 115° instead of 110°. The anhydrous morphine is then dissolved in $N/10$ acid and the excess titrated with $N/10$ alkali instead of directly titrating with acid as formerly.

It is highly probable that the ninth revision of the U. S. P. will abandon the old and cumbersome modified Squibb method in favor of a lime process differing from the Steven's method (Vol. VI, 425) chiefly in the extraction of the opium with water and treatment of this previously concentrated extract with lime, instead of mixing the opium and lime before extracting with water. This method will compare very favorably with the B. P. method in speed and accuracy.

Lyons[2] makes a careful and interesting comparison of the Steven's and eighth revision U. S. P. methods. The new U. S. P. standard will be based on anhydrous instead of crystallised morphine, and for powdered opium is 10% to 10.5% as against 12% to 12.5% in the eighth revision.

A new variation of the lime process suggested by Guerin[3] consists in using a small amount of acetone mixed with the solution from which the morphine is precipitated and in washing the morphine crystals with a saturated solution of the same in acetone. In view of the solubility of morphine in mixtures of water and acetone, as determined by Guerin (page 496), this does not seem advisable and is certainly not so good as the use of ether.

La Wall[4] finds that lactose interferes seriously with the U. S. P., eighth revision, assay, unless titration of the morphine is used, and Debourdeaux reports the same for the lime method (see page 507).

Carles,[5] in an apparent endeavour to obtain thorough precipitation by long standing without the risk of having the morphine contaminated with calcium meconate or its double salt with ammonium, precipitates the calcium as oxalate from the lime solution filtered from the opium and then precipitates the morphine with crystallised sodium carbonate. ·

[1] *Pharm. J.*, 1913 (4), **37**, 876.
[2] *J. Am. Pharm. Assoc.*, 1915, **4**, 92.
[3] *J. pharm. chim.*, 1913, **7**, 162.
[4] *J. Am. Pharm. Assoc.*, 1912, 411.
[5] *Rep. Pharm.*, 1912, **24**, 97.

In this connection attention should be called to the possibility of morphine forming crystalline compounds with sodium or potassium carbonate; hence the use of ammonia as a precipitant is preferable.

Dohme[1] suggests a process of estimating morphine in opium and a number of its preparations which resembles that of Anneler for pantopon (page 502); it depends on retaining the morphine in the aqueous solution, made strongly alkaline with sodium or potassium hydroxide, as an alkali morphinate while the other alkaloids are removed by ether, subsequently making acid and then alkaline with ammonia and extracting the morphine with a chloroform-*iso*butyl alcohol mixture. No analytical results indicating the success of the process were given. Van der Wielen[2] did not find the Anneler process satisfactory on opium.

Normal Opium.—The feeling that estimation of morphine alone does not correctly indicate the medicinal value of opium has been growing in recent years, so Van der Wielen (*loc. cit.*) advocates a so-called "normal opium" standardised for its four most important constituents, recommending as a standard the following: morphine, 12%; narcotine, 6%; codeine, 1%; and meconic acid, 5%. He estimates the morphine by some standard lime method (B. P. preferred), the narcotine and codeine by the process given in Vol. VI, p. 393, and the meconic acid colourimetrically as follows:

Macerate 1 grm. of opium with 100 c.c. of water for 24 hours, shaking occasionally; filter, and with 25 c.c. of filtrate, representing 0.25 grm. of opium, mix 5 c.c. of 25% solution of basic lead acetate, let stand at least 15 minutes and filter, washing the precipitate with water till the washings are colourless. Dissolve the precipitate in warm N/10 hydrochloric acid making up to exactly 100 c.c., which will give a slightly yellowish solution. As a comparison standard dissolve 0.05 grm. of meconic acid in N/10 hydrochloric acid to make exactly 250 c.c. adding sufficient Orange G. to give a colour identical with that of the solution from opium. A colourimetric comparison of the two solutions may now be made, using 1 drop of ferric chloride test solution to 5 c.c. of each solution to produce the characteristic colour with meconic acid, and employing any suitable colourimeter.

Estimation of Morphine in Compounds.—The accurate determination of morphine in galenical compounds is often extremely difficult, and while in fluids such as camphorated tincture of opium or even Sydenham's laudanum reasonably good results are obtainable if especial care is used, in a preparation such as the U. S. P. Compound Licorice Mixture, results by any process so far devised are very unreliable even though duplicates may be concordant. The writer is aware that considerable work has been done on the assay of this mixture and that some analysts believe it possible to determine the morphine with fair accuracy, but so far all actual results he has seen do not substantiate this belief.

[1] *J. Amer. Pharm. Asso.*, 1915, 4, 85.
[2] *Yearbook of Pharm.*, 1913, 443.

A process applicable to acid opium liquors and designed especially for Sydenham's laudanum is given by Debourdeaux[1] and involves the following considerations. Morphine is soluble in 100 parts of water saturated with carbon dioxide, but a solution of morphine in lime water precipitates both calcium carbonate and morphine when treated with carbon dioxide. If now a lime water solution from opium, containing the morphine, is treated with the gas the calcium carbonate and morphine are precipitated, but on continued treatment to complete saturation the morphine redissolves.

To 150 c.c. of Sydenham's laudanum add an excess of freshly slaked lime, dilute the mixture with water to 300 c.c. and allow to stand for about half an hour, shaking frequently or continuously; filter and wash the precipitate with 50 c.c. of water. The precipitated magma is now mixed with 150 c.c. of water, the mixture again filtered and the precipitate washed with three 50 c.c. portions of water. The whole mixed filtrate is now saturated with carbon dioxide, filtered if necessary, the precipitate washed with water saturated with the gas, and the filtrate and washings evaporated to 100 c.c., cooled, 50 c.c. of ether and 10 c.c. of $N/1$ ammonia added, and after thorough shaking the whole set aside for 24 hours. The precipitated morphine is washed with water saturated with morphine and ether, dried at 100° and then mixed with 5 grm. of slaked lime and 118 c.c. of water. After solution of the crystals, filter and to an aliquot part (preferably 80 c.c., representing 100 c.c. of the original laudanum) add 10% by volume of alcohol, 50% of ether and 2% of ammonium chloride. Let stand 24 hours after thorough shaking, collect the crystals, wash and dry as before, wash again with petroleum ether, dry at 100° and weigh. (Instead of weighing, as recommended, it would be preferable to titrate.) Add the correction for morphine in the mother liquors, as determined by LeClere,[2] of 31 mg. for each 50 c.c. of the final solution used for precipitation.

The estimation of morphine in plain tablets or pills, while not usually difficult, offers some probable complications. If a lime method is used any starch present must be removed by filtering the solution of the tablet and treating the sediment with water and acid before lime is added, otherwise some morphine will be absorbed by the starch in the alkaline solution (see Debourdeaux).[3] Lactose, glucose, gum and dextrin also may interfere. With regard to lactose, see page 505. The best procedure is therefore to use some approved shake-out method, and though a number of these have been described in the literature, for lack of space only one will be fully given here with brief mention of other similar methods, the one selected being essentially that of Williams.[4]

Dissolve a sufficient number of the tablets to give about 0.1 to 0.2 grm. of morphine in a few c.c. of water acidified with hydrochloric acid. If any in-

[1] J. Pharm. Chim., 1913, 8, 424.
[2] J. Pharm. Chim., 1913, 7, 521.
[3] J. Pharm. Chim., 1913, 8, 301.
[4] Am. J. Pharm., 1914, 86, 308.

soluble matter is present, filter and wash the precipitate thoroughly with dilute hydrochloric acid and water and evaporate the filtrate to a volume of about 10 c.c. If the tablets are completely soluble, keep the volume of the solutions to about 10 c.c. Transfer to a separating funnel and add 15 c.c. of a mixture of 2 volumes of chloroform and 1 volume of alcohol and then sufficient 10% ammonia to make distinctly alkaline; agitate carefully and thoroughly for several minutes and draw off the chloroform solution through a pledget of cotton. Repeat this extraction twice more and then use three 10 c.c. portions of chloroform alone. Evaporate the combined chloroform extracts to dryness, add a few drops of alcohol and again evaporate. Dissolve the residue in 10 c.c. of $N/10$ acid and titrate with $N/50$ alkali, using cochineal as indicator.

A similar process, using three extractions with 50 c.c. portions of amyl alcohol, was suggested by Bernegau and Heidlberg in a paper before the Pennsylvania Pharmaceutical Association in 1912 which process was modified by E'we and Vanderkleed.[1] Anneler's method applied to pantopon (see page 502) may also be used to advantage and has been found very useful by Englehardt.[2] Thorburn[3] uses as a solvent 3 volumes of phenylethyl alcohol and 1 volume of benzene.

In tablets containing no diluent except cane or milk sugar, simple solution in water, precipitation with ammonia, and weighing or titration of the washed and dried crystals is quite satisfactory.

Reference may be made to the work of Adams and Doran[4] on smoking opium and its composition, showing variations in morphine from about 8% to 17%.

Pott[5] found that some undecomposed morphine sublimed with opium smoke at atmospheric pressure, this being detected by its action on the respiratory centres of rabbits and by the biological reaction on mice.

Toxicology of Opium and Morphine.—Investigation by Rosenbloom[6] of the organs of a cadaver 13 months after burial with marked signs of putrefaction showed no substances giving reactions of morphine or other alkaloids, nor was there evidence of any substances interfering with the usual processes of estimating morphine.

Grutterink and Van Ryn[7] in the course of some investigations found that $2\frac{1}{2}$ years after death it was possible to show definitely the presence of morphine in both the stomach and liver, though only minute quantities were found in the liver. Identification was made by various colour reactions and by actual separation of crystals of morphine. Doepmann[8], in order to test carefully the stability of morphine and the value of toxicological

[1] *J. Am. Pharm. Assoc.*, 1913, 979.
[2] *Ap. Ztg.* (*New York*), 33, 141.
[3] *J. Ind. Eng. Chem.*, 1911, 3, 754.
[4] *J. Ind. Eng. Chem.*, 1912, 4, 429.
[5] *Biochem. Zeit.*, 42, 67.
[6] *J. Biol. Chem.*, 18, 131.
[7] *Pharm. Weekblad.*, 1915, 52, 423.
[8] *Chem. Zeit.*, 1915, 39, 69.

examinations long after putrefaction has set in, mixed varying amounts of morphine hydrochloride with chopped horse flesh and examined the mixture at intervals of from 1 to 11 months. He was able to isolate and obtain characteristic reactions of morphine in every case, even when as little as 20 mgm. had been added to each kilo of flesh and after standing for 11 months.

ERRATA IN VOL. VI.

Page 353, line 4, for page 354 read page 407.
Page 365, title, for "sovlents" read "solvents."
Page 389, line 9, for "carbonate" read "bicarbonate."

STRYCHNOS ALKALOIDS.

By CHARLES E. VANDERKLEED.

The many species of the genus *Strychnos* have a widely vaiying physiolog-ical action, according as they contain more, less, or in some cases no strych-nine or brucine. A. F. Sievers[1] tabulates those species of *Strychnos* which have thus far been reported on as to their content of strychnine and brucine.

The seeds of *Strychnos Nux Vomica* continue to afford the principal source of the alkaloids strychnine and brucine. The Madras and Bombay districts of India, and the Saigon district of Cochin China are the commercial geographic sources of this drug. In none of these districts has the cultivation of the nux vomica tree become a business. The trees grow in a wild state, attain-ing a height of 30 to 40 ft., and a circumference of 3 to 4 ft. Their growth and development depend largely on the extent of the rainfall in the district to which they are indigenous. The young trees, however, in their wild state are in some cases attended and manured by the natives, and in such cases they come into bearing in 10 to 12 years. When it is desired to cultivate the nux vomica it is customary to plant the young seedlings in large tubs or pots of rich, loose soil, which must be kept well watered. Poor results have thus far been obtained when raised or cultivated as garden or plantation trees. The seeds are harvested from March to July.

Oil of Nux Vomica.—In the preparation of extract of nux vomica a small quantity of heavy fixed oil sometimes separates and may be skimmed off. This oil is dark brown in colour and has a specific gravity of about 0.956 at 25°. When subjected to the following assay process, a sample of this oil yielded 4.66 grm. of strychnine per 100 c.c.

Dissolve 10 c.c. of the oil in 40 c.c. of ether and shake out with three por-tions of 5% sulphuric acid. Make alkaline with ammonia, shake out with chloroform and complete the assay by the U. S. Pharmacopœia method for the assay of the drug.

Test for Brucine in Strychnine.—D. B. Dott[2] condemns the use of undi-luted nitric acid in testing strychnine for the presence of brucine. He advocates the use of a 1 : 1 nitric acid stating that this will detect 0.01% of brucine in strychnine. He recommends for pharmacopœial use the following test: "When 0.05 grm. of the strychnine in powder form is dissolved at the ordi-nary temperature in 4 c.c. of acid prepared by mixing equal volumes of nitric acid and water, the colour should, after 5 minutes, be purely yellow, showing

[1] *Midland Druggist and Pharm. Rev.*, 1911, 45, 233.
[2] *Pharm. Jour.*, 89, 144-171.

no red or orange tinge." In using the nitric acid method for the estimation of strychnine in the presence of brucine, Dott employs 1 c.c. of concentrated nitric acid and 10 c.c. of diluted sulphuric acid for every 0.25 grm. of brucine. On standing for 10 minutes at ordinary temperature all brucine is destroyed. If kept at 40°, however, an appreciable amount of strychnine is destroyed.

Species	Synonym or common name	Habitat	Parts used	Constituents (strychnine and brucine)	Uses, remarks, etc.
S. Nux vomica Linn.	Poison nut, false angostura bark	Tropical India	Seed, bark and stem	Seeds, strychnine 0.25–2 % brucine 0.50–2 % Bark, brucine (anhyd.) 7.78 % Wood, brucine (anhyd.) 2.26 % Leaves, brucine (anhyd.) 0.33 %	Medicinal
S. Ignatii Berg.	St. Ignatius bean	Philippine Islands	Seeds	Strychnine, 0.52–1.5 % Brucine, 1.43 %	Medicinal
S. Colubrina Linn.	Serpent's wood	East Indies	Wood	Wood, 0.96 % total alkal. Bark, 5.54 % total alkal. (Both strychnine and brucine present)	Antidote for snake bites and medicinally.
S. Rheedii Clarke	Serpent's wood	Malabar	Wood and leaves	Wood and bark—strychnine and brucine Seeds, 0.06 % brucine	Antidote for snake bites and medicinally
S. potatorum Linn.	Clearing nut	Ceylon, E. Indies	Seeds	Traces of brucine	Clarifying water, also medicinally
S. guianensis Mart.		Guiana	Bark	Strychnine and brucine	Arrow-poison (currarine, a poisonous alkaloid constitutes the poison). Medicinally
S. Tieute Leschen		Java	Root-bark	Root-bark—strychnine. Leaves and seeds—about 1.4 % strychnine and trace of brucine	Arrow-poison
S. pseudochina St. Hill.		Brazil	Bark	None	Intermittent fever
S. ligustrina Zipp.		Malayan Archipelago	Bark and wood	Bark—7.78 % anhyd. brucine	
S. laurina Wall.			Wood	Leaves, none	
S. monosperma Miq.		Java		None	
S. malaccensis Benth.		Cochin-China	Bark	Brucine	Hydrophobia
S. javanica		Cochin-China	Bark	2.7 % brucine	Hydrophobia
S. suaveolens Gilg.		West Africa	Wood	Brucine	
S. Icaja Baill.		Africa	Wood	Strychnine in bark, leaves and roots	Arrow-poison
S. toxifera		Guiana	Bark	None	Arrow-poison
S. aculeata		Africa	Seeds	Strychnine, none; brucine, about 0.05 % mostly in kernels	Fish-poison (poisonous action due to a glucoside)
S. Quaqua Gilg.		East Africa	Fruits	Seeds—strychnine, none; brucine, trace	Fruit (pulp) used for food
S. spinosa Lam.		East Africa	Fruit	Seeds—trace	Fruit (pulp) used for food
S. Tonga Gilg.		East Africa	Fruit	Seeds—trace	Fruit (pulp) used for food

The Assay of Nux Vomica and Its Preparations.—*Method of the United States Pharmacopœia.*—Having concluded that in the hands of many operators the assay of nux vomica and its preparations for strychnine only, by the nitric acid method, is liable to lead to erroneous results, and having also been satisfied that the proportion of strychnine to brucine in nux vomica seeds does not greatly vary, the Revision Committee of the United States Pharmacopœia have chosen for the forthcoming issue (Ninth Revision) a method for total alkaloids, adopting as a standard for the drug not less than 2.5%, for the fluid extract not less than 2.37 nor more than 2.63 grm. total alkaloids in 100 c.c., and for the tincture not less than 0.237 nor more than 0.263 grm. total alkaloids in 100 c.c.

The process for the *drug* consists in extracting by maceration with a mixture of chloroform and ether, with ammonia water to liberate the alkaloids, taking an aliquot part, extracting with diluted sulphuric acid, rendering alkaline with ammonia, extracting with chloroform, evaporating, weighing, and titrating against $N/10$ sulphuric acid, using the average molecular weight of strychnine and brucine as the basis for the calculation. The *fluid extract* is diluted with ammonia water and shaken out, following the same method as used for the drug. 100 c.c. of the *tincture* are evaporated to 10 c.c. and assayed in the same way as the fluid extract.

Method of the British Pharmacopœia, 1914.—In the new British Pharmacopœia, 1914, the ferrocyanide method of assay of nux vomica preparations (see Vol. VI, p. 473) of the 1898 edition has given way to the nitric acid method. For the *drug*, the method is essentially as follows:

7.5 grm. of nux vomica in No. 60 powder are macerated with frequent shaking for half an hour with a mixture of 25 c.c. of chloroform, 50 c.c. ether, and 5 c.c. ammonia. 50 c.c. of the clear liquid (representing 5 grm. drug) are transferred to a separator and shaken out with three portions each of 10 c.c. of $N/1$ sulphuric acid solution. The combined acid solutions are rendered alkaline with ammonia and shaken out with three portions of chloroform.

The chloroform is recovered from the combined solutions, and the alkaloidal residue is dissolved in a mixture of 5 c.c. of diluted sulphuric acid and 10 c.c. water. The solution is warmed to 50° and 3 c.c. of a mixture of equal volumes of nitric acid and water are added to oxidise and destroy the brucine. After 10 minutes the solution is made alkaline with solution of sodium hydroxide and shaken out with three portions of chloroform. The mixed chloroform solutions are washed with 5 c.c. of water and evaporated to dryness in a tared dish. 5 c.c. of alcohol are added and evaporated, and the residue of strychnine alkaloid dried at 100° and weighed. The standard for nux vomica is not less than 1.25% of strychnine as determined by the above method.

The *liquid extract* is assayed by taking 10 c.c., evaporating to a syrupy extract, dissolving in 20 c.c. of water, rendering alkaline with a solution

of 5 grm. sodium carbonate in 25 c.c. water, and shaking out with chloroform, after which the method for the drug is followed as outlined above. The standard for liquid extract of nux vomica is not less than 1.45 grm. nor more than 1.55 grm. of strychnine in 100 c.c.

The *dry extract* is assayed by exhausting 3 grm. with 70% alcohol and testing the alcoholic solution by the process as outlined for the liquid extract. The standard for dry extract of nux vomica is not less than 4.8 grm. nor more than 5.2 grm. of strychnine in 100 grm.

The *tincture* is assayed by the process described under the liquid extract. The standard for tincture of nux vomica is not less than 0.120 grm. nor more than 0.130 grm. of strychnine in 100 c.c.

CINCHONA ALKALOIDS.

By OLIVER CHICK.

Cinchona Ledgeriana Seeds.—Previous investigators have failed to find any alkaloids in the seeds of *C. Ledgeriana*, *C. officinalis*, or *C. succirubra*. P. van Leersum[1] however, by the following process of extraction has obtained up to 0.38% of total alkaloids on the dried seeds of *C. Ledgeriana*. The seeds, after powdering, were extracted with petroleum ether to free them from fat, the powder was dried, reground, mixed with slaked lime and sodium hydroxide solution, and extracted with benzene. The mixed alkaloids were extracted from the benzene by hydrochloric acid.

Cuprea Bark.—Two parcels of this bark, now seldom seen, offered for sale this year (1914), and examined in Messrs. Howards and Sons' laboratory, were found to contain no cupreïne.

Titration of Cinchona Alkaloids.—Richter[2] states that the Fifth German Pharmacopœia (1910) method does not give concordant results, and that Poirrier's blue and lacmoid are better indicators to use than hæmatoxylin. He recommends the following acid method of extraction and estimation. 3.75 grm. of powdered bark are warmed on the water-bath with 2.5 c.c. of 25% hydrochloric acid and 20 c.c. of distilled water for 15 minutes. When cold, 30 grm. of chloroform and 60 grm. of ether are added, and the whole shaken. 10 c.c. of 10% sodium hydroxide solution are now added, and the mixture again shaken during 15 minutes. The ethereal layer is then cleared by the addition of 1 grm. of powdered tragacanth and 0.5 grm. of ignited magnesia. The chloroform-ether extract is filtered, and 60 grm. shaken out with 20, 20 and 10 c.c. of $N/10$ hydrochloric acid, and then with 10 c.c. of water. The organic solvents are driven off, and the aqueous portion made up to 250 c.c. 100 c.c. of this are treated with 20 c.c. of water and 30 c.c. of $N/10$ picric acid solution. When the precipitate has settled, 50 c.c. of clear liquid are filtered off and titrated with $N/10$ potassium hydroxide solution, using phenolphthalein as indicator.

R. Gaze[3] also finds that the method prescribed in the Fifth German Pharmacopœia for the estimation of alkaloids in cinchona barks is unreliable in the case of those of high alkaloidal content, more chloroform-ether mixture and sodium hydroxide solution being required than therein stated; moreover, the temperature of extraction is important as more alkaloid is extracted at 30° than at 15°.

[1] *Pharm. Weekblad.*, 1913, **50**, 1464–1467.
[2] *Apoth.-Zeit.*, 1912, **27**, 949–950, 960–961.
[3] *Apoth.-Zeit.*, 1913, **28**, 144–147.

Constitution.—The constitution of the cinchona alkaloids, although not yet known with certainty, is becoming better understood each year, thus enabling chemists to convert certain of these alkaloids into others closely related to them. The isomerism in this group is now known to be due to the spatial arrangement around the asymmetric carbon atom marked (b) in the following formula for cinchonine, since the asymmetry of the other three carbon atoms (a), (c), and (d) may be destroyed without interfering with the isomerism of the products.

$$
\begin{array}{ccc}
 & (c) & (d) \\
CH_2{-}CH & - CH & -CH = CH_2 \\
\; | & | & | \\
\; | & CH_2 & | \\
\; | & | & | \\
\; | & CH_2 & | \\
\; | & | & | \\
C_9H_6N{-}{-}CHOH{-}{-}{-}{-}CH{-}N & - CH_2 \\
(a) & (b) &
\end{array}
$$
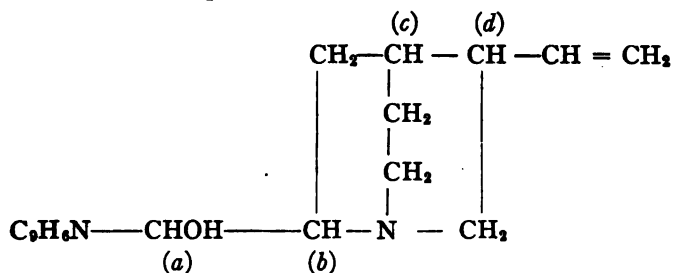
Following up the conversion of cinchonicine into cinchonine by Rabe,[1] Kaufmann and Huber have converted hydrocinchonicine (hydrocinchotoxine) into a mixture, which on careful examination showed the presence of about 50% hydrocinchonine and 10% hydrocinchonidine.[2]

It has been found possible to prepare the hydrocinchona alkaloids in nearly theoretical yield by the hydrogenation of the alkaloids under pressure in the presence of palladium black as catalyst (see page 519). Every other method of hydrogenation invariably splits the second half of the molecule, yielding amorphous gummy bodies which do not give crystalline salts.

Rabe and his pupils[3] have started some preliminary experiments on the synthesis of cinchona alkaloids which may be watched with great interest.

Dobbie and Fox[4] have confirmed the presence of the 6-methoxyquinoline nucleus in quinine, and the 6-hydroxyquinoline nucleus in cupreïne by means of their absorption spectra, which are practically identical with the absorption spectra of 6-methoxyquinoline and 6-hydroxyquinoline.

General Properties of Cinchona Alkaloids.—G. N. Watson[5] gives the following test for *cinchona alkaloids*. A freshly prepared alcoholic solution of α-naphthol is treated with a little strong sulphuric acid (2 drops to 1 c.c.). When a few drops of this reagent are added to an aqueous solution of quinine sulphate a yellow precipitate is produced, soluble in excess of the reagent to a yellow solution. Sulphates of quinidine, cinchonine, and cinchonidine, but no other white alkaloids, give this yellow colour, which may be obtained with 1 c.c. of a 1 : 2000 solution of quinine sulphate.

[1] *Ber.*, 1908, **41**, 67; 1911, **44**, 2088.
[2] *Ber.*, 1913, **46**, 2913.
[3] *Ber.*, 1912, **45**, 2163.
[4] *Trans.*, 1912, **101**, 77–81.
[5] *Amer. J. Pharm.*, 1913, **85**, 502.

Quinine.—J. Ville[1] states that anhydrous crystals of quinine are easily obtained by blowing air through a wash-bottle containing ammonia into a solution of from 1 to 20% strength of quinine hydrobromide heated on the water-bath.   G. L. Schaefer[2] has obtained anhydrous quinine in the form of slender needle-shaped crystals by allowing the gelatinous mass obtained by dissolving quinine hydrate in a small amount of acetone to stand for several days.

Detection and Estimation of Quinine.—A new method of applying the thalleioquin test for quinine is given by C. H. LaWall,[3] the following reagent being required: 0.5 grm. of potassium bromate dissolved in 10 c.c. of 10% hydrobromic acid is made up to 100 c.c.   100 c.c. of the quinine solution, which may be as weak as 1 : 200,000, are placed in a Nessler cylinder and 5–10 drops of the bromine reagent added, when a green tint is obtained.   In testing drugs or extracts, about 1 grm. of the material is shaken with ammonia and 20 c.c. of ether, the ether extract being evaporated to dryness.   The residue is taken up with 1 c.c. of $N/10$ sulphuric acid, and made up to 15 c.c. with water.   5 c.c. are then placed in a Nessler cylinder, made up to 100 c.c. and tested as above.   Should no green tint be obtained the remaining 10 c.c. may be tested similarly.   Should no green colour be now produced the cinchona alkaloids which give the thalleioquin reaction (see Vol. VI, p. 507) are absent, or present in amount to less than 0.1 mg.

C. Mannich and L. Schwedes[4] state that pyramidone (1-dimethyl-amino-2 : 3-dimethylpyrazolone) prevents the thalleioquin reaction, a red colour being obtained instead of a green.   Pyramidone also interfers with the herepathite reaction.   It may be separated from quinine by shaking out the bases from an alkaline solution, and washing the extract thoroughly with water, the pyramidone being removed in the water.

P. J. Kruysse[5] gives a method for the separation of quinine from other cinchona alkaloids by precipitation as nitroprusside, but gives no indication of the accuracy of the method.

T. Cockburn and J. W. Black[6] have published a method for the estimation of quinine in certain organic liquids, which gives excellent results if the details of manipulation are strictly adhered to.   In testing urine, 250 c.c. of the sample are made strongly alkaline with sodium hydroxide, warmed, and extracted three times with ether (at as high a temperature as practicable).   The combined ether extracts are washed with water, placed in a flask, the ether evaporated, and the residue dried in the steam-bath.   It is dissolved in 20 c.c. of anhydrous ether and the solution filtered into a weighed 4 ounce conical flask, with the ethereal washings of the flask and filter.   With the flask is weighed a filtering tube, consisting of a small thistle-funnel with a

[1] *Bull. Soc. Chim.*, 1912, 11, 398.
[2] *8th Int. Cong. Appl. Chem.*, 1912, Sect. VIII B, 75–84.
[3] *Amer. J. Pharm.*, 1912, 84, 484.
[4] *Apoth. Zeit.*, 1912, 27, 343.
[5] *Chem. and Drug.*, 1913, 82, 34.
[6] *Analyst*, 1911, 36, 396.

10-mm. bulb packed with asbestos. To the ethereal solution are added 10 c.c. of saturated ethereal solution of citric acid, and after 24 hours the liquid is filtered through the tube by suction, and the precipitate washed three times with 10 c.c. of ether which is similarly drawn off through the filter tube. Filter and flask are now dried, at first at a low temperature, finally at 100°, and weighed. The process may be used for the estimation of quinine in the presence of caffeine, but not for the separation of quinine from other cinchona alkaloids.

Salts of Quinine.—The British Pharmacopœia, 1914, has substituted the Kerner test for the methods of testing quinine salts given in the 1898 edition. In doing so it has raised the standard of purity of these salts from products containing 8 to 9% of other alkaloidal sulphates to products containing about 3% cinchonidine sulphate together with 1 to 2% hydroquinine sulphate (*i.e.*, the normal amount in quinine sulphate).

Quinine Sulphate.—The British Pharmacopœia, 1914, has changed Dr. Paul's gravimetric test of the 1898 Pharmacopœia for Prof. Kerner's volumetric test. The details of the test are exactly similar to the British Pharmaceutical Codex test except that the quinine sulphate is dried at 50° (until only two mols. of water of crystallisation remain) before weighing out the 2 grm. of the salt for the test (see Vol. VI, pp. 520–521). 6 c.c. of ammonia, sp. gr. 0.959 are allowed.

Quinine Hydrochloride.—The British Pharmacopœia, 1914, gives the following modified Kerner test for quinine hydrochloride. "2 grm. are dissolved in a warm mortar in 20 c.c. of water at 60°, 1 grm. of powdered non-effloresced sodium sulphate added, the mixture triturated, cooled, allowed to stand at exactly 15° for half an hour with occasional stirring, the crystals of quinine sulphate pressed and the expressed liquor filtered. 5 c.c. of this filtrate, transferred to a dry test-tube and brought to a temperature of 15° yield, on the gradual addition of 6 c.c. of solution of ammonia, also at a temperature of 15°, a precipitate which redissolves on rotating the tube."

It may be noted that the results obtained with samples of quinine hydrochloride by the above test bear no relation whatever to the results obtained by the ordinary Kerner test on samples of quinine sulphate of similar purity (see Vol. VI, p. 528). Moreover, 2 grm. of quinine hydrochloride contain 12% more alkaloid than 2 grm. of quinine sulphate.

N. Tarugi[1] has devised a fairly rapid method for detecting more than 3% of cinchonine and cinchonidine hydrochlorides in quinine hydrochloride. 25 c.c. of the cold quinine hydrochloride solution are saturated with sodium nitrate, allowed to stand for 3 hours, filtered and 5 c.c. of the filtrate treated with 1.5 c.c. of ammonia (sp. gr. 0.880) when should cinchonine or cinchonidine be present to the extent of more than 3% a turbidity is produced.

Quinine Dihydrochloride.—The official salt of the 1898 British Pharmacopœia was one having the formula $C_{20}H_{24}O_2N_2,2HCl,3H_2O$ which should

[1] *Gazzetta*, 1914, 44, I, 131–151.

contain 12% of water; but, as was pointed out in Vol. VI, p. 528, the salt met with in commerce contains only 3 to 5% of water. The 1914 Pharmacopœia recognises this, giving the formula as B,2HCl and allowing a 3% water-content. It states that 1 grm. of the salt dissolved in 20 c.c. of water requires for neutralisation not more than 5.0 c.c. of normal sodium hydroxide solution with phenolphthaleïn as indicator. The Kerner test is carried out on this salt as for the hydrochloride except that the salt is dissolved in 15 c.c. of water and 5 c.c. of normal sodium hydroxide solution. The same remark applies to this Kerner test as to that for the hydrochloride; and the alkaloid content of the dihydrochloride is half way between that of the sulphate and the hydrochloride.

Quinine Formate.—Hampshire and Pratt[1] having examined several commercial samples of quinine formate find that it is a monohydrate of the formula $C_{20}H_{24}O_2N_2,HCOOH,H_2O$, and is not anhydrous as is generally stated.

Quinine Glycerophosphate.—Rogier and Fiore[2] describe quinine glycerophosphate $(C_{20}H_{24}O_2N_2)PO_3,OC_3H_7O_2,H_2O$ as white needles becoming somewhat yellow on exposure to light. It melts at 180°–181° and is soluble in 78 parts of boiling water; $[\alpha]_D^{210} = -133°\ 33'$ (0.2474 grm. of anhydrous salt in 20 c.c. of 90% alcohol), $[\alpha]_D^{17} = -140°\ 24'$ (0.2093 grm. in 20 c.c. of absolute alcohol).

Citrate of Iron, Quinine, and Strychnine, Easton's Syrup, Etc.—While the total alkaloids in these preparations may be estimated with ease and accuracy by the ordinary methods, no satisfactory method for the separation of the quinine and strychnine existed before the modification of the ferrocyanide method lately proposed by Simmonds.[3] Excellent results are obtained if the details of the process are adhered to. The strychnine must not exceed 0.1 grm. in the 50 c.c. of acid used; and it has been found in Messrs. Howards and Son's laboratory that not more than 3 hours should be allowed for the ferrocyanide precipitation to prevent excessive co-precipitation of the quinine. Having obtained and weighed the total alkaloids in the usual way (from 5 to 10 c.c. of Easton's syrup, and 5 grm. of citrate of iron, quinine and strychnine are convenient quantities to take), these are dissolved in 50 c.c. of 10% sulphuric acid. 5 c.c. of 4% potassium ferrocyanide solution are run in from a burette drop by drop, stirring well, and the mixture set aside (3 hours). The precipitate is filtered through a small filter (7 cm.) and washed lightly with about 3 c.c. of 5% sulphuric acid. With the aid of about 10 c.c. of 10% ammonia solution and a fine jet of water the precipitate is washed into a small separator and extracted three times with chloroform, using 15, 10 and 5 c.c. The chloroform solutions are collected in another separator, and the alkaloids extracted from them with 50 c.c. of 20% sulphuric acid, using 30, 10, and 10 c.c.; then the precipitation and other operations are repeated as before, until

[1] *Pharm. J.,* Special Issue, 1913, 26.
[2] *Bull. Sci. Pharmacol.,* 1913, 20, 72.
[3] *Analyst.* 1914, 39, 81–83.

the chloroform extracts are again obtained. The chloroform is evaporated carefully, a little alcohol being added towards the end to prevent spluttering, and the residue of strychnine weighed after drying it for an hour or so at 100°. The quinine may be taken by difference between the total alkaloid and strychnine, or by bulking the two acid filtrates, making alkaline, and extracting with chloroform or ether.

Aristoquinine and Euquinine.—Aristoquinine is usually called carbonate of quinine. Biginelli,[1] however, regards it as carbonylquinine $(C_{20}H_{23}O_2N_2)_2$-CO, having prepared the true anhydrous carbonate B_2,H_2CO_3, m. p. 168°–169°, by passing carbon dioxide into a solution of quinine in aqueous ether (see Vol. VI, p. 59). Similarly euquinine is the ethyl ester of quinine-carbonic acid, and not ethylcarbonate of quinine. Were these bodies merely salts of quinine, alkalis would liberate the alkaloid from them; this, however, is not the case, even hot alkalis being without action on them. Moreover, they are rapidly hydrolysed by boiling dilute acids, after which treatment the quinine may be recovered by making the solutions alkaline and extracting with ether.

Angeloni[2] gives the solubility of aristoquinine as 0.20% in ether, and of euquinine as 3.33% in ether.

Hydroquinine.—In view of the increased demand for hydroquinine sulphate and the difficulty of its economical manufacture from commercial quinine sulphate by the acid sulphate and permanganate method (see Vol. VI, p. 533, footnote), the following new method of preparation by hydrogenation of quinine sulphate is of great importance.

"One part of palladium black is added to a solution of 250 parts of quinine sulphate in 1,400 parts of water and 40 parts of sulphuric acid, and the mixture is shaken with hydrogen under a low pressure until it is stable towards permanganate: after filtration the solution is neutralised whilst hot, when hydroquinine sulphate will at once crystallise in the form of slender needles."[3]

No methods have been given for testing the purity of hydroquinine sulphate, but the following have been used by the writer. No mineral matter should be left on gentle ignition. No ammonium salts should be indicated on boiling a little of the cold water washings of the salt with sodium hydroxide. 1 grm. of the salt dissolved in 20 c.c. of dilute sulphuric acid and cooled to 0° by means of ice, should not immediately discharge the colour from 1 drop of 1% permanganate solution (test for non-hydrogenated alkaloids). The alkaloid obtained by passing through ether and ammonia and taking to dryness should dissolve almost completely in a little chloroform (test for hydrocinchonidine). The optical rotation may also be taken (Vol. VI, p. 500).

The author has estimated the hydroquinine content of many samples of

[1] *Annali. Chim. Appl.*, 1914, **1**, 397–400.
[2] *Boll. Chim. Farm.*, 1913, **52**, 675.
[3] *Eng. Pat.* 3948, Feb. 16, 1912.

quinine sulphate from different sources this year (1914), and has found in all cases from 1 to 2%.

Cinchonine.—It has been found in Messrs. Howard and Son's laboratories that many commercial samples of cinchonine hydrochloride contain from 2 to 4% of sodium chloride. This impurity is probably due to the method of preparation, as it is found that salt crystallises persistently with cinchonine hydrochloride. Any sodium chloride present may be detected by dissolving some of the cinchonine hydrochloride in chloroform, the inorganic salt being insoluble. To estimate this impurity it is best to incinerate a weighed quantity of the substance at as low a temperature as possible, wash out the sodium chloride from the thoroughly charred mass on a filter, and estimate the chloride in the filtrate by means of silver nitrate.

ERRATA. CINCHONA ALKALOIDS, VOL. VI.

Page 494, line 25, for "page 592," read "page 492."

Page 494, line 28, for "page 493," read "page 490."

Page 497, line 33, for "Parrier's blue as indicated," read "Poirrier's blue as indicator."

Page 499, line 17, for "conguinamine," read "conquinamine."

Page 517, line 7, for "$c = 22$," read "$c = 2.2$."

Page 533, line 17, for "page 199," read "page 512."

Page 535, line 9, for "page 194," read "page 500."

Page 535, line 22, for "tolerabl," read "tolerably."

Page 536, line 27, for "quinine," read "quinidine."

Page 542, line 4, for "pages 520 and 521," read "pages 522 and 525."

BERBERINE AND ITS ASSOCIATES.

By EDWARD HORTON.

Berberine, $C_{20}H_{17}O_4N,H_2O$; or $C_{18}H_{11}(OCH_3)_2O_2N,H_2O$.

Since the publication of Vol. VI, berberine has been synthesised by Pictet and Gams,[1] and converted into hydrastinine by Freund.[2]

Reactions and Detection.—Rosenthaler and Görner[3] state that a characteristic behaviour of berberine is that its solutions gelatinise when treated with dinitro-α-naphtholsulphonic acid.

Senft[4] has described a method of detecting hydrastine and berberine microscopically in the seeds of *Hydrastis canadensis*. The powdered endosperms are extracted with ether which removes the fat and the hydrastine. The ethereal extract is evaporated and the residue extracted with alcohol, which dissolves the alkaloid. After filtering and concentrating the alcoholic solution, it is treated with excess of iodine solution, when a coarse-grained black precipitate is formed, which after some time is transformed into small rosette-like groups of crystals. The endosperm after treatment with ether is extracted with hot alcohol which dissolves the berberine. The evaporated filtrate gives all the reactions for berberine described by Tunmann (*Beiträge zur Mikrochemie einiger Wurzeldrogen, Gehes Handelsberichte*, 1912). That with zinc chloriodide solution is very characteristic. If some of this reagent is allowed to flow under the cover-glass so as to mix with an aqueous berberine solution, an immediate cloudiness is formed, small crystalline flocks rapidly separate which quickly grow into dense rosettes of extraordinarily thin needles. Side by side with these, single needles and sheaves of needles are observed.

Estimation: Gravimetric Method.—Richter[5] recommends the following method: The alcoholic extract of Berberis bark representing 4 grm. of the drug, or an equivalent amount of the tincture, is evaporated to dryness, the residue dissolved in water and 10 c.c. of 15% sodium hydroxide solution added. The whole is shaken with 80 grm. of ether for 10 to 15 minutes and 1 grm. of powdered tragacanth added. 40 grm. of the ethereal solution are treated with 40 grm. of $N/10$ solution of picrolonic acid (nitrophenylmethyl*iso*nitrosopyrazolone) and the precipitate of berberine picrolonate collected on a Gooch crucible, washed with a little ether and alcohol, dried at 110° and weighed.

[1] *Compt. rend.*, 1911, 153, 386; *Ber.*, 1911, 44, 2480.
[2] *Annalen*, 1913, 397, 30.
[3] *Zeitsch. anal. Chem.*, 1910, 49, 340.
[4] *Pharm. Post.*, 1913, 46, 977.
[5] *Arch. Pharm.*, 1914, 252, 192.

The weight obtained multiplied by 0.561 gives the equivalent amount of berberine.

David[1] has proposed a new method for the estimation of berberine in hydrastine extracts. The berberine and hydrastine are precipitated with potassium bismuth iodide, and the hydrastine separated by extraction with ethyl acetate. The precipitate is then treated with 10% sodium hydroxide solution and the berberine extracted with a mixture of equal parts of ether and chloroform.

Hydrastine, $C_{21}H_{21}O_6N$; or $C_{19}H_{15}(OCH_3)_2O_2N$.

Reactions and Detection.—Baroni and Borlinetto,[2] who have studied a reaction of calomel with some alkaloids, state that when equal parts of hydrastine hydrochloride and mercurous chloride are triturated together in the presence of water a yellow colouration is immediately produced. A similar behaviour is shown by quinine dihydrochloride, but in this case a brown colour results. Senft's method of detecting hydrastine microscopically is described under Berberine (page 521).

Mayrhofer[3] recommends picrolonic acid as a suitable reagent for the microchemical detection of hydrastine and berberine in plants. The plant section is treated with a mixture of 2 parts of saturated picrolonic acid solution 1 part of glycerol and 1 part of alcohol. At a dilution of 1 in 30,000 berberine sulphate gives crystals in the form of bright yellow clusters, which become brown or black when treated with iodine tincture mixed with an equal volume of glycerol. At a dilution of 1 in 15,000 hydrastine hydrochloride forms crystals, mostly bright yellow in colour, but the smallest may be colourless. They are not altered by treatment with iodine.

Estimation: Gravimetric Methods.—The British Pharmacopœia, 1914, gives the following process of estimating hydrastine in Liquid Extract of Hydrastis.

"Transfer 10 c.c. of the liquid extract to a 100 c.c. graduated flask, add 20 c.c. of a solution of potassium iodide diluted with 60 c.c. of water, and then sufficient water to produce 100 c.c. Shake the mixture for several minutes and filter. Transfer 50 c.c. of the filtrate to a separator, render alkaline with solution of ammonia, add 30 c.c. of ether and shake at intervals during several minutes. Allow the liquids to separate, draw off the aqueous portion into a beaker and the ethereal portion into a tared beaker. Return the aqueous solution' to the separator, and repeat the operation with two successive portions, each of 20 c.c., of ether for 1 minute. Draw off and reject the aqueous layer; transfer the ethereal solutions to the tared beaker and evaporate at a gentle heat; dry the residue on a water-bath and weigh. The weight is that of the hydrastine in 5 c.c. of the liquid extract examined.

"Examined by the foregoing process liquid extract of hydrastis is found to contain in 100 c.c. 2 grm. of hydrastine. Limit of error 0.1 grm. in excess or defect."

The method for the estimation of hydrastine in fluid extracts described in the Dutch Pharmacopœia (4th Edition) is inaccurate in some respects accord-

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[1] *Pharm. Post.*, 1915, **48**, 1.
[2] *Giorn. Farm. Chim.*, 1911, **6b**, 241.
[3] *Pharm. Post.*, 1914, **47**, 547.

ing to van der Haar,[1] who recommends the following process: 10 grm. of the extract are boiled in a large flask with 20 c.c. of water until the weight is reduced to 10–11 grm: 1.5 c.c. of hydrochloric acid are added, and, after cooling, water is added sufficient to make the total weight up to 20 grm. After shaking with 1 grm. of powdered talc the whole is filtered. 10 c.c. of the filtrate are shaken for 1 minute in a 100 c.c. flask with 4 c.c. of ammonia and 25 c.c. of ether, 25 c.c. of light petroleum and 1.5 grm. of powdered tragacanth are then added, and the whole is vigorously shaken until the liquid becomes clear. 40 c.c. of the ethereal layer are transferred to a flask, another 5 c.c. of light petroleum added, and distilled until 35 c.c. have passed over. The flask is then kept in a cool place for 18 to 24 hours, and after pouring off the mother liquor and washing the crystals with 2 c.c. of light petroleum, these are dried on a water-bath and weighed.

The Fifth German Pharmacopœia[2] describes the following processes for the assay of *Hydrastis* drugs.

Hydrastis Rhizome (not formerly standardised).—This is extracted with ether and ammonia for 3 hours, and an aliquot part of the ethereal solution filtered off. The ether is distilled from the filtrate, the solid residue taken up with dilute acid, the solution filtered and the filter washed with water. The acid liquid is then made alkaline with ammonia and the alkaloid shaken out with ether—once only; an aliquot part of the ethereal solution is evaporated and the residue weighed. This is the only case (besides the fluid extract of the same drug) where the alkaloid is weighed and not titrated, and nearly every stage of the process shows a departure from the usual procedure. The requirement is that the alkaloid so obtained shall correspond with 2.5% in the drug, but the amount actually present is probably greater than this, having regard to the final shaking out of the alkaloid with only one portion of ether.

Fluid Extract of Hydrastis.—This is diluted with water and then evaporated to remove all alcohol, acidified with hydrochloric acid, the liquid well shaken with talc and filtered. An aliquot part of the filtrate is made alkaline with ammonia and shaken with ether for some minutes, light petroleum is then added and the shaking repeated. After addition of a considerable amount of powdered tragacanth the liquid is further shaken until the ethereal layer is clear. An aliquot part of the latter is filtered off, evaporated and the alkaloid weighed. The amount found is required to correspond with 2.2% in the fluid extract, which therefore does not quite correspond with the rhizome in strength. In this case an intermediate stage of purifying the alkaloid by shaking into acid and back again, has been omitted, being presumably rendered superfluous by the use of acid in the first place, which, with the addition of talc and filtering, is new. The use of tragacanth in separating the ether-petroleum layer is also new, and the latter contains a much larger proportion of light petroleum. The strength is somewhat increased, being formerly 2%.

[1] *Pharm. Weekblad.*, 1911, 329.
[2] *Pharm. J.*, 1911, 86, 295.

Rupp[1] describes a method similar to that of the German Pharmacopœia. Gsell's method[2] of estimating hydrastine and berberine in hydrastis extract depends on the determination of the amount of methoxyl in the respective alkaloids.

David[3] states that for the estimation of hydrastine the methods given in the German and Belgian Pharmacopœias are the most trustworthy, then follows that of the Austrian Pharmacopœia, whilst the French, Dutch, Swiss, and U. S. A. methods are untrustworthy, as the hydrastine obtained by their use is very impure. The U. S. A. method may be improved by removing the alcohol before the hydrastine is extracted with ether. In the estimation of hydrastine, alcohol must be removed previously, berberine should be separated by precipitation with potassium iodide or hydrochloric acid, and the ethereal extract must be mixed with light petroleum, and the mixed solution treated with tragacanth.

Hydrastinine, $C_{11}H_{13}O_3N$.

Reactions.—Reichard[4] gives a large number of reactions for hydrastinine. The two following are the most characteristic and establish the identity of the alkaloid. A few particles of the hydrochloride are triturated with a minute crystal of potassium ferro- or ferricyanide and a drop of water is then added. With ferricyanide a splendid reddish-green precipitate is obtained, which is characterised by its dichroism. The crystals form elongated prisms and are beautifully developed. When held up to the light they appear bluish dark green with total reflection of the light; the colours are permanent. With the ferrocyanide, crystals are obtained resembling mother of pearl; these are particularly characteristic. Sodium nitroprusside gives a compound not unlike that obtained with the ferricyanide. The behaviour of the alkaloid with sulphuric acid is also noteworthy as the yellow colour produced on warming disappears on cooling, and the test may be repeated over and over again.

[1] *Apoth. Zeit.*, 1909, **24,** 922.
[2] *Chem. Zeit.*, 1914, **38,** 541.
[3] *Pharm. Post.*, 1915, **48,** 1.
[4] *Pharm. Zentr-h.*, 1911, **52,** 1253.

CAFFEINE, TEA AND COFFEE.

By J. J. FOX AND P. J. SAGEMAN.

Very little additional information concerning the various xanthine derivatives or the constituents of tea and coffee has been published since the article in Vol. VI went to the press. As was perhaps to be expected, most of the new work concerns the methods of analysis, and the usual number of "new" methods for the estimation of caffeine has appeared. We have taken advantage of this opportunity to draw attention to the difficulties which, in our opinion, attend the estimation of caffeine.

There appears to be a useful field for work in the investigation of the constituents of tea and coffee. Much of the best information available rests upon data obtained many years ago and has not hitherto been revised.

Purine Bases.—*Identification of purine derivatives by microchemical methods.*[1] Mercuric chloride reacts with those purine bases which contain at least 1 methyl group forming characteristic precipitates. Caffeine and theophylline give rosettes of crystals, while with theobromine smaller masses of crystals result. According to Wagenaar so small a quantity as 1 μ of caffeine can be detected by breathing on the dried precipitate. Antipyrine-caffeine (*migrainine*) does not give the reaction.

According to Camilla and Pertusi[2] xanthine bases may be readily detected in the following manner. The extracted basic compounds are treated with a few drops of concentrated potassium hydroxide solution. A saturated solution of potassium permanganate is then added drop by drop and the mixture warmed. In the presence of xanthine bases gas is evolved. An ódour of carbylamine is also said to be observed. (It is, however, difficult to see how carbylamine could be formed in such a case. The only odour detected by the writers was that of methylamine, a normal product of the oxidation of caffeine).

Many new pharmaceutical preparations of purine bases have been prepared recently. A neutral compound of caffeine with aminoacidylphenetidine is stated to be suitable for subcutaneous injections. It is, however, readily split into its components by alkalis. Acidyl compounds of theobromine with stronger acidic properties than the alkaloid are obtained by treatment of metallic salts of theobromine with acid haloids.

C. O. Johns[3] has prepared isomers of theobromine and caffeine by alky-

[1] Wagenaar. *Chem. Zentralb.*, 1914, 1, 1026.
[2] *Chem. Zentralb.*, 1912, 2, 1581.
[3] *J. Biol. Chem.*, 1914, 17, 1.

lating with dimethylsulphate.   2 : 8-Dihydroxy-1 : 9-dimethylpurine yields
2 : 8-dihydroxy-1 : 7 : 9-trimethylpurine, melting at 240°.   It is more sol-
uble in water than caffeine and does not give the colouration with chlorine
water and ammonia characteristic of the latter substance.   2 : 8-Dihydroxy-
1-methyl purine similarly gives 2 : 8-dihydroxy-1 : 7-dimethylpurine.   This,
on further methylation produces the above-mentioned isomer of caffeine.

Assay of Caffeine Sodium Salicylate.—The following method is taken from
the British Pharmaceutical Codex.   1 grm. of the drug is dissolved in 20 to
25 c.c. of water and sufficient aqueous sodium hydroxide is added to make
the solution alkaline.   The liquid is then thoroughly shaken 3 times with
chloroform, using successively 15, 10, and 10 c.c.   The chloroform solution
is evaporated to dryness and the caffeine is weighed.   Caffeine sodium sal-
icylate should contain at least 40% of caffeine.   Lehmann and Müller[1] rec-
ommend the addition of powdered gum tragacanth to the alkaline solution in
order to ensure the complete extraction of the caffeine.

Estimation of Caffeine in Tea, Coffee, Etc.—From the fact that several
investigators have thought it necessary to reexamine the processes for the
estimation of caffeine in natural products, it would appear that in many cases
difficulty has been experienced in applying the methods described in the lit-
erature on the subject.   It has already been indicated (Vol. VI, pp. 590–591,
606 and 612) that it is necessary to employ some preliminary treatment in
order to decompose those compounds (such as caffeine-potassium chlorogen-
ate) in which the caffeine occurs naturally, before the alkaloid can be
completely extracted.

In the case of tea, boiling with water is as a rule sufficient to extract the
alkaloid, but with coffee, kola, etc., acid or alkaline treatment is generally
necessary in order to ensure the complete liberation of the base.   It is ap-
parent that the preliminary treatment should be of such a nature as to leave
the material in a condition that will permit of ready permeation by a solvent
suitable for the extraction of the caffeine.   In our opinion, disregard of this
point is frequently responsible for the difficulties that seem to beset the esti-
mation of caffeine.   It is not easy otherwise to explain the divergent results
obtained by various workers who have used processes which appear to differ
only in minor details.   In most cases it is probable that the preliminary
treatment is sufficient to decompose the caffeine compounds and apparently
the failure to extract the caffeine completely is due either to the unsuitable
condition in which the material is left for extraction or to the choice of unsuit-
able solvents.   Murray[2] compares Gorter's process[3] with that of Lendrich
and Nottbohm.[4]   In the former process 11 grm. of the coffee are moistened
with 3 c.c. of water, allowed to stand for 30 minutes and then extracted with
chloroform for 3 hours; in the latter 20 grm. of coffee are treated with 10 c.c.

[1] *Apoth. Zeit.*, 1911, 26, 647.
[2] *J. Ind. Eng. Chem.*, 1913, 5, 668.
[3] *Annalen*, 1908, 358, 327
[4] *Zeit. Nahr. Genussm.*, 1909, 17, 241.

of water for 2 hours and then extracted with carbon tetrachloride for 3 hours. The subsequent purification of the extract was of such a nature as to lead to no appreciable loss of caffeine in either case. Murray, however, found that Gorter's method gave much higher results than that of Lendrich and Nottbohm.

As regards coffee we have found that Katz's method, as described on page 609, Vol. VI, gives concordant and accurate results. We prefer the extraction by continuous agitation of the treated coffee with chloroform to extraction in a Soxhlet apparatus, as there is then no doubt that the chloroform is in intimate contact with every part of the finely ground coffee. All workers know the difficulties which arise in extracting a compact moist mass with an immiscible solvent.

Zöller[1] pointed out that caffeine is not affected when heated at 100° with concentrated sulphuric acid, and suggested a method for the estimation of caffeine in tea, involving a preliminary heating at 100° with sulphuric acid. Allen made numerous experiments in order to devise a satisfactory method based on Zöller's principle, but in his experience the process had the objection that caffeine is retained by the charred mass and very prolonged and repeated extractions were necessary to ensure the complete removal of the caffeine.

Costes[2] has recently applied the same principle to the extraction of caffeine in coffee. He recommends the following procedure: 20 grm. of ground coffee are mixed with 15 to 20 c.c. of sulphuric acid (D. 1.835) and heated on the water-bath for 10 to 15 minutes, after which the mixture is extracted with 3 successive quantities of boiling water (200, 150, and 100 c.c.) boiling for 10 minutes after each addition of water. The acid extract is filtered into a basin containing sodium hydroxide solution insufficient in quantity to neutralise all the acid. The solution is made alkaline with sodium carbonate, rapidly evaporated to 250 c.c. and extracted 3 times with chloroform, using successively 50, 35, and 30 c.c. The chloroform extract is evaporated to about 4 c.c., mixed with 2 c.c. of sulphuric acid and heated for 10 minutes on the water-bath. The residue is dissolved in water, filtered, made ammoniacal, cooled and extracted with chloroform. The chloroform is distilled off and the residue weighed. It usually contains not more than 93% of pure caffeine and it is therefore desirable to estimate the nitrogen by Kjeldahl's method and calculate the nitrogen as caffeine. This method is claimed to be especially suitable for caffeine-freed coffee.

In whatever manner the caffeine may have been extracted from coffee, tea or kola it is desirable to make certain that the final extract as weighed is pure caffeine. The most satisfactory check is the determination of the nitrogen by Kjeldahl's method in the weighed residue. Where time is an important factor the caffeine as first extracted may be purified in the following manner suggested by Lendrich and Nottbohm (loc. cit.): The residue is treated at

[1] Zeit. Anal. Chem., 12, 106.
[2] Anal. Chim. Anal., 1912, 17, 246.

the ordinary temperature with 10 to 30 c.c. of 1% potassium permanganate. After 15 minutes the excess of permanganate is decomposed by adding, drop by drop, a 3% hydrogen peroxide solution containing 1% of acetic acid. The liquid is then heated on a water-bath for 15 minutes, cooled, filtered, and washed thoroughly. The caffeine is extracted from the filtrate by chloroform in the usual manner.

Toxicity of Caffeine.—W. Salant and J. B. Rieger[1] describe the results of a large number of experiments on rabbits, guinea-pigs, cats and dogs. They found the fatal dose to range from 0.14 to 0.35 grm. of caffeine per kilogram of body weight, varying with the mode of administration. Cats and dogs were more susceptible than rabbits or guinea-pigs. Caffeine is not cumulative in dogs and rabbits.

Tea.

Sawamura's recent investigations[2] led to the following important conclusions as to tea:[3]

(1) In steaming tea leaves it is desirable to limit the steaming so that only the oxidising enzymes are destroyed. This may be effected by steaming for 30 seconds only. The remaining enzymes play an important part in the first stage of rolling tea leaves and it is probable that the production of a fine aroma is dependent on their action.

(2) Green tea is improved in quality by 1 hour's firing at 70°, a higher temperature spoiling the flavour and colour.

(3) The optimum temperature for black tea is 80°.

(4) Refiring is accompanied by a decrease in both the caffeine and tannin.

(5) The operation of rolling crushes the cells and the liberated juices dry on the surface of the leaves. As a consequence, the proportion of readily soluble constituents of the tea is increased.

In view of the widespread opinion that the ratio of caffeine to tannin is an important factor in the valuation of various classes of tea it is interesting to note the patent which has recently been taken out for the adjustment of the ratio between these two constituents by the addition of one or other to tea. It is proposed to make the addition of the necessary constituent by atomising a solution of the substance into the air currents during the drying process (English patent, 10,471, May 2, 1912).

Moisture in Tea.—For ordinary purposes the estimation of moisture by heating at 100°–105° under atmospheric pressure gives sufficiently accurate results. In order, however, to avoid inaccuracy due to oxidation, etc., it is preferable to use some method of drying in an inert atmosphere or at reduced pressure. This may be secured either by standing the material in a vacuum desiccator over concentrated sulphuric acid at a pressure of 15

1 *U. S. Dept. Agric., Bureau of Chem., Bull.* 148.
2 *Inter. Cong. Appl. Chem.,* 1912, 18, 313.
3 For recent work on "The Fermentation of Tea" and the effect of treatment on the quality of the leaf, see Mann, *Reports to the Indian-Tea Association, Calcutta.*

mm. for a long period, or alternatively, by heating at 98° *in vacuo*. The
A. O. A. C. recommend either drying in a current of hydrogen or *in vacuo*
for approximately 5 hours in a water-oven.

The use of calcium carbide in estimating moisture has been proposed by
F. H. Campbell.[1] The carbide is mixed with a weighed quantity of the
sample in a special apparatus and the loss of weight calculated to water,
using a factor obtained by treating a portion of the same carbide with a
known weight of water. In nearly all cases this method gave higher results
than drying either in air or by heating under reduced pressure.

Tannin in Tea.—H. L. Smith[2] has worked out a method for the estimation
of tannin in tea which is a modification of Chapman's cinchonidine method
for the estimation of tannin in hops. It depends upon the complete precipi-
tation of tannin by a saturated solution of cinchonine sulphate, the cincho-
nine tannate being weighed. A similar method involving the use of quinine
was described by Tatlock and Thomson. This method is dealt with in Vol.
VI, p. 619. Smith's method is briefly as follows: 10 grm. of tea are boiled
with 800 c.c. of water for half an hour, filtered and washed with 200 c.c. of
water. The filtrate is cooled and made up to 1,000 c.c. Of this solution 50
c.c. are treated with chloroform to remove the caffeine, and the aqueous liquid
is concentrated to about one-third its bulk. 50 c.c. of saturated cinchonine
sulphate solution are added to the hot liquid which is then put aside for some
hours. The precipitated cinchonine tannate is filtered on a Gooch crucible
which has previously been washed with half saturated cinchonine sulphate
solution and dried at 100°. The precipitate is also washed with half saturated
cinchonine sulphate solution and thoroughly dried by suction. The crucible
is dried in a vacuum desiccator over sulphuric acid and then heated at 100°
till constant in weight. This preliminary drying is necessary in order to
avoid fusion of the precipitate. The dried precipitate contains 55% of
tannin. The method gives results higher than those given by Procter's
modification of Löwenthal's method. We have already indicated (Vol. VI,
p. 615) that this is due to imperfect knowledge of the nature of the tannin
in tea. The results obtained by gravimetric methods for its estimation show
that the adoption of an arbitrary factor for the permanganate solution on
the assumption that the tannin in tea is gallo-tannic acid is in general
in accurate.

Smith points out the necessity of removing caffeine before precipitation
of the tannin, owing to the co-precipitation of caffeine with cinchonine
tannate and the impossibility of removing the caffeine from the precipitate by
washing.

Detection of Added Colouring Matter in Tea.—In view of the prohibition
of the entry into the United States of America of artificially coloured teas
E. Alberta Read[3] has described the following simple tests for the detection of

[1] *J. Soc. Chem. Ind.*, 1913, 32, 67.
[2] *Analyst*, 1913, 38, 312.
[3] *8th Int. Cong. App. Chem.*, 1912, 18, 301.

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added colour. The method allows of the handling of a large number of samples in a short time, thus avoiding undue delay at the place of import. 20 to 25 grm. of tea are placed on a sieve of 16 to 24 meshes to the cm., crushed slightly and then shaken over a piece of white paper. The dust on the paper is crushed by means of a spatula. This procedure streaks the paper and the added colour can be detected, especially with the aid of a lens. Good light is essential for this work.

The usual chemical tests for Prussian blue, indigo, ultramarine and turmeric can be applied. Carbon (graphite) is readily identified by its appearance under the microscope.

As regards Prussian blue, West[1] suggests pressing the powdered tea between sheets of filter paper moistened with oxalic acid solution. On drying the paper and brushing off the tea, blue spots indicate Prussian blue. The natural colouring matter of tea does not stain the paper.

Coffee.

The question of the aroma of roasted coffee has received renewed attention by Bertrand and Weisweiller.[2] 5 kilos of freshly roasted ground coffee were distilled in a current of steam and the aqueous distillate concentrated to 20 c.c., which contained the whole of the volatile constituents. These 20 c.c. consisted of a heavy oil and an aqueous layer. The aqueous layer was neutralised with hydrochloric acid, filtered and treated with barium silicotungstate. The resulting precipitate was recrystallised from water and dried. It was found to consist of pyridine silicotungstate. 200 to 250 mg. of pyridine per kilo were yielded by various samples of commerical roasted coffee. Pyridine, according to Bertrand and Weisweiller, is an essential factor in the production of the aroma of coffee. If the volatile oil be added alone to sweetened water the characteristic aroma of coffee is not produced unless a corresponding quantity of pyridine is also added.

Moisture in Coffee.—The standard method of the A. O. A. C. as described under tea is the most satisfactory for accurate work.

A recent prosecution in England raised an interesting point in connection with the limits of moisture permissible in coffee and chicory mixtures. Hodgson[3] is of opinion that 6% of moisture is too low a maximum limit having regard to the hygroscopic character of ground coffee and chicory mixtures To test the point Hodgson placed samples of chicory, coffee, and a mixture of chicory and coffee in a drawer and determined the moisture at intervals. He found that in 21 days the increase in moisture was for coffee from 2.0 to 9.7, chicory from 11.3 to 15.9, and for a mixture of chicory and coffee (66% of coffee) from 3.5 to 11.5%. The conditions were described as "similar to those under which coffee and chicory are usually kept in retail shops," but it is very doubtful whether coffee mixtures are generally kept in this manner.

[1] *J. Ind. Eng. Chem.*, 1912, **4**, 528.
[2] *Compt. Rend.*, 1913, **157**, 212.
[3] *Analyst*, 1913, **38**, 454.

Chlorogenic Acid.—Gorter[1] describes a delicate and characteristic reaction for chlorogenic acid in coffee and other plants. The substance is boiled with dilute hydrochloric acid for 1 hour, and extracted with ether. The washed extract is concentrated and a dilute solution of ferric chloride is added. A violet colour indicates chlorogenic acid. By this means Gorter has demonstrated the presence of chlorogenic acid in *Ficus Elastica* and *Castilloa Elastica*.

Caffeol.—Grafe[2] examined 3 kinds of coffee in order to trace the source of the caffeol: (1) ordinary coffee; (2) caffeine-free coffee which was prepared by treatment with superheated steam followed by benzene; and (3) coffee purified by scrubbing in warm water. No. (2) yielded much less caffeol and also much less crude fibre than Nos. (1) and (3). Grafe concludes that the caffeol has its origin in the crude fibre, the amount of which in (2) was much diminished by the treatment to which it has been subjected.

Toxic Substances in Coffee.—Certain acidic constituents of coffee are reputed to have a toxic effect and according to several recent patents these may be removed by roasting the berries with clay or kaolin, preferably with the addition of magnesia. The exact value of treating coffee in this manner is not clear to the authors.

Caffeine-free Coffee.—Numerous patents have recently been taken out for removing the greater part of the caffeine from coffee. The most interesting of these depends on the low solubility of caffeine in very cold water. The beans in this case are first extracted with very cold water to remove extractives other than the xanthine alkaloids; the latter are then extracted with hot water. The beans are then made to re-absorb the previously concentrated cold water extract, dried, and roasted.

Coffee Substitutes.—In addition to the substances already enumerated in Vol. VI, p. 670, special attention has lately been called to the use of the seeds of *Lathyrus sativus* (a genus of leguminosæ) largely grown in Southern Europe and known as Gesse or Jarosse. Patents have been granted for the use of Soja beans (French patent) and for grains such as rye roasted with juniper berries (English patent). The detection of these materials in admixture with roasted coffee may be readily effected by the aid of the microscope.

The estimation of cereal substitutes in coffee by means of the alkalinity of the ash is recommended by Rozsenyi.[3] He found that the acid required to neutralise the ash of 5 samples of coffee from different sources closely averaged 50 c.c. of $N/1$ acid per 100 grm. of roasted coffee. The ash from 100 grm. of roasted barley, wheat, or rye required about 1 c.c. of $N/1$ acid. These figures form the basis for the calculation of the proportion of coffee in mixtures of coffee with roasted cereals. The examples given by Rozsenyi of the analysis of made up mixtures of coffee and roasted cereals show close agreement with the quantities actually present. These values for the alkalinity of the ash agree with those which we have nearly always found for coffee. In fact

[1] *Rec. Trav. Chim.*, 1912, **31**, 281.
[2] *Monatsh.*, 1912, **33**, 1389.
[3] *Chem. Zeit.*, 1913, **37**, 1482.

we have always considered this determination a valuable one when judging the purity of a sample. Rozsenyi's method forms a valuable adjunct to the microscopical examination, decoction value, etc., of mixtures of coffee with roasted cereals. He also points out that unground roasted coffee may be extracted with water for the preparation of coffee extract, and the exhausted beans dried, glazed and sold as fresh coffee. By this treatment, however, the coffee loses 40% of its mineral matter and the adulteration may be detected by the abnormally low ash of the sample.

Detection of Chicory Extract in Decoctions of Coffee and Chicory.— LaWall and Forman[1] recommend a determination of the cupric reducing power as a means of detecting the presence of chicory extract in coffee extracts. According to these observers the extractives of roasted coffee contain 1.92 to 2.64% of reducing sugars, while the extractives of roasted chicory contain 25.2 to 27.7%. The test is not affected by the presence of cane sugar which is detected polarimetrically. More than 3% reducing sugars calculated on the extractives indicates the presence of chicory extract (see also Tatlock and Thomson, *J. Soc. Chem.*, *Ind.*, 1910, **23**, 138).

Coffee Extracts.—With regard to the examination of coffee essences and extracts for preservatives, Lythgoe and Marsh[2] point out that the ethereal extract of pure coffee essence gives a buff-coloured precipitate with ferric chloride solution and this may be mistaken for the precipitate given by benzoic acid. The ammonium salt of this substance, however, differs from ammonium benzoate in forming insoluble compounds with salts of manganese, nickel, magnesium, calcium, barium, and strontium. The following test for benzoic acid is based on this difference. Extract the acidified coffee solution with ether and shake the ethereal layer with dilute ammonia solution. Evaporate the ammoniacal solution to small bulk, add manganese sulphate solution, filter, and test the filtrate for benzoic acid with ferric chloride.

Kola.—Galenical preparations of kola are rather difficult to assay for caffeine owing to the formation of persistent emulsions when the preparations are shaken with chloroform and alkali. According to G. Meillière[3] the formation of such emulsions may be avoided by the use of the following method. 2.5 grm. of kola extract or 25 grm. of the fluid extract evaporated to small bulk are dissolved in 25 c.c. of simple syrup, transferred to a 250 c.c. separating funnel and treated with 2.5 grm. of potassium hydrogen carbonate. The froth produced by the brisk effervescence is easily broken down by adding chloroform of which 10 to 20 times the volume of the syrup is used in successive portions for the extraction. The chloroform solution is filtered and evaporated for alkaloids.

François,[4] on the other hand, recommends triturating with magnesia,

[1] *Amer. J. Pharm.*, 1913, **85**, 535.
[2] *J. Ind. Eng. Chem.*, 1911, **3**, 842
[3] *J. Pharm. Chem.*, 1912, **5**, 438.
[4] *J. Pharm. Chim.*, 1913, **8**, 411.

drying over sulphuric acid, and extracting with chloroform. It is doubtful (see Vol. VI, p. 591) whether the extraction would be complete by this method.

ERRATA IN VOL. VI.

Pages 580 and 581. The last line but one of page 581 should be transferred to page 580, so as to read as the second line from the bottom on this page.

Page 592, line 16 from bottom, 896 should read 1896.

Page 595, line 14, 38.5% should read 38.1%.

Page 647. Coffearine is said by Gorter (*Annalen*, 1910, **372**, 239) to be identical with trigonellin.

Page 662. Mr. A. E. Johnson informs the editors that the method of estimating the percentage of coffee in coffee mixtures attributed to him is really due to E. W. Jones, who used it in obtaining the results recorded in the *Analyst*, 1882, **7**, 76, in the case of the Birkenhead "Coffee" samples. The mistake has arisen in consequence of the method having been originally given in the First Edition of Johnson's "Analyst's Laboratory Companion."

OTHER VEGETABLE ALKALOIDS.

By GEORGE BARGER, M. A., D. Sc. AND A. J. EWINS, D. Sc.

Alkaloids of Colchicum.

The colloidal nature of aqueous solutions of colchicine has been shown by Zeisel and Stockert[1] to be only apparent. Determination of the molecular weight in acetic acid or in boiling ethylene dibromide gave normal values for the formula $C_{22}H_{25}O_6N$. In cold ethylene dibromide or in water somewhat higher values were obtained.

Zeisel and Stockert[2] have obtained the following new bromo-derivatives:

A *monobromide*, $C_{22}H_{24}O_6NBr,MeOH$, crystallised from methyl alcohol, m. p. 151–155° (133–135° in sealed capillary). It is obtained by treating a dilute aqueous solution of colchicine with one molecular proportion of hydrobromic acid.

A *dibromo-derivative*, $C_{22}H_{25}O_6NBr_2$, m. p. 146–150° (125°, sealed capillary) produced in the presence of an excess of hydrobromic acid.

A *tribromo-colchicine*, $C_{22}H_{22}O_6NBr_3$, m. p. 131° (118–122° in sealed capillary) is obtained when a methyl alcoholic solution of colchicine is treated with excess of bromine.

Tribromo-colchiceine, $C_{21}H_{20}O_6NBr_3,H_2O$, and tribromotrimethyl-colchicinic acid, $C_{19}H_{18}O_5NBr_3$, were also obtained.

The physiological action of some derivatives of colchicine has been investigated by Fühner.[3]

In descending order of activity they may be arranged as follows: 1. colchicine (trimethylacetylcolchicinic acid); 2. trimethylcolchicinic acid methyl ether; 3. trimethylcolchicinic acid: 4. colchiceine (dimethylacetylcolchicinic acid). Replacement of the acetyl group of colchicine by benzoyl gives rise to a product the activity of which is about one-tenth that of colchicine. Oxycolchicine, $C_{22}H_{23}O_7N$, m. p. 266–268[4] obtained by the action of chromic acid on colchicine has an action on frogs, similar to that of veratrine, but has no action on mammals.

Alkaloid of Laburnum and Furze.

Cytisine.—The constitution of cytisine has been recently further investigated by Ewins.[5] It had previously been found by Freund and his collaborators[6] that on treatment with hydriodic acid and phosphorus at

[1] *Monatsh.*, 1913, 34, 1327.
[2] *Monatsh.*, 1913, 34, 1339.
[3] *Arch. exp. Path. Pharm.*, 1913, 72, 228.
[4] *Monatsh.*, 1913, 34, 1181.
[5] *Trans.*, 1913, 103, 97.
[6] *Ber.*, 1901, 34, 615; 1904, 37, 16; 1906, 39, 814.

about 230°, cytisine yielded among other products (a) a feebly basic crystalline solid *cytisoline*, $C_{11}H_{11}ON$, m. p. 198°, which ,on reduction with sodium and alcohol gave a basic oil, α-cytisolidine $C_{11}H_{15}N$ and (b) a basic oil, β-cytisolidine, which was considered by Freund to be isomeric with α-cytisolidine.

It has now been shown, however, (Ewins, *loc. cit.*) that β–cytisolidine has the composition $C_{11}H_{11}N$ and this base was identified, by comparison with the synthetic product, as 6 : 8-dimethylquinoline. Further α-cytisolidine, $C_{11}H_{15}N$, was shown to be the corresponding tetra-hydro derivative, namely, 6 : 8-dimethyltetrahydroquinoline. Cytisoline, $C_{11}H_{11}ON$, from which α-cytisolidine is produced on reduction, must, therefore, be a hydroxy-6 : 8-dimethylquinoline. It is not phenolic in character, nor is it identical with 2-hydroxy-6 : 8-dimethylquinoline and must, therefore, be either 3, or 4-hydroxy-6 : 8-dimethylquinoline.

Cytisoline, $C_{11}H_{11}ON$, differs in composition from cytisine, $C_{11}H_{14}ON_2$, only by the elements of ammonia. On this account it is suggested that cytisine may be formed by the fusion of three rings (benzene-pyridine-pyrazole) somewhat as shown
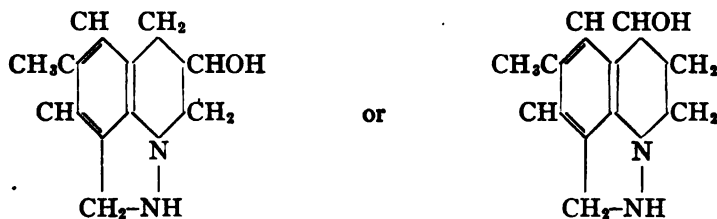
A compound so constituted might conceivably lose ammonia to form dimethylquinoline derivatives, but there is no direct evidence that cytisine has this constitution.

N-Methylcytisine, $C_{12}H_{16}ON_2$, hitherto only obtained by methylation of cytisine has recently been found[1] to occur naturally in the rhizome and roots of *Caulophyllum Thalictroides* (Linné) Michaux (Nat. Ord. Berberidaceæ) a plant indigenous to North America. For the isolation of the alkaloid the powdered material was first completely extracted with alcohol, the bulk of the solvent removed by distillation and the dark coloured viscid residue distilled with steam. The aqueous distillation residue was then purified by shaking thoroughly with amyl alcohol and with ether and finally precipitated with a slight excess of basic lead acetate. After removal of the excess of lead the liquid was made alkaline with sodium hydroxide and repeatedly extracted with chloroform. The residue after removal of the chloroform was converted into the hydrochloride, which was obtained crystalline from a mixture of ethyl acetate and alcohol. From the pure hydrochloride the base was obtained by dissolving in water and extracting with chloroform after making alkaline with sodium hydroxide. On evaporation of the chloroform, the

[1] Power and Salway, *Trans.*, 1913, 103, 194.

base solidified and was recrystallised from benzene and light petroleum. The yield from 22 kilos of material was about 5 grm. (0.23%).

Methylcytisine forms colourless prismatic needles m. p. 137°. It is readily soluble in water, alcohol, chloroform, and benzene, and is optically active, $[\alpha]_D = -221.6°$ in aqueous solution. The *hydrochloride*, B, $(HCl)_2$, H_2O forms colourless prisms, m. p. 250 – 255° (decomp.). The *aurichloride*, B, $HAuCl_4$, crystallises in golden yellow needles m. p. 205° (decomp.) and the *picrate* forms long yellow needles m. p. 228° (after sintering from 220°).

For the *assay* of the alkaloid in the drug the following procedure was found to give reliable and consistent results.

20 grm. of caulophyllum in No. 60 powder were treated with 100 c.c. of chloroform and 10 c.c. of a 10% aqueous sodium carbonate solution, and the mixture was vigorously shaken from time to time during 4 hours. The mixture was filtered and 50 c.c. of the filtrate transferred to a separating funnel and shaken with 10 c.c. of N/10 sulphuric acid. The acid liquid was separated and the chloroform again shaken with 10 c.c. of N/10 sulphuric acid. The acid solutions were mixed, extracted with 20 c.c. of ether and, after separating the ether, 5 c.c. of 10% sodium carbonate solution were added. The alkaline liquid was extracted three times with successive quantities of 20 c.c. of chloroform; the extracts were combined, washed twice with water (2 c.c. each time) and the chloroform was distilled off. The residual alkaloid was dissolved in 10 c.c. of N/50 sulphuric acid, 10 c.c. of ether were added and the excess of sulphuric acid was determined by titration with N/50 barium hydroxide (iodoeosin indicator). Two estimations gave 0.086% and 0.078% respectively.

According to Laidlaw (quoted by Power and Salway, *loc. cit.*) methy-cytisine is very similar in its action to cytisine,[1] but is very much less active, the effect on the blood-pressure of the cat being about one-tenth as powerful as that of cytisine.

The Alkaloids of Calabar.

Physostigmine (*Eserine*).—Further work on the constitution of this alkaloid[2] has shown that eserine, $C_{15}H_{21}O_2N_3$, when heated *in vacuo* at 150° decomposes, giving eseroline, $C_{13}H_{18}ON_2$, which can then be distilled without undergoing any decomposition at a temperature of 240°. Eseroline yields a very characteristic benzoate, B, C_6H_5COOH, which crystallises in leaflets, m. p. 155–156°, and is obtained by adding solid benzoic acid to an ethereal solution of the base. Eseroline *picrate*, B, $C_6H_3O_7N_3$, appears to be dimorphous, usually melting at 167–168°, but occasionally at 190–191°.

By the distillation of methyl eserolinium carbonate in a good vacuum (oil pump) a distillate is obtained which can be separated into two products:

(1) a basic compound which is believed to have the constitution denoted by the formula

[1] Dale and Laidlaw, *J. Pharmacol. Exp. Therap.*, 1912, **3**, 205.
[2] Straus, *Annalen*, 1913, **401**, 350.

$$\text{OH.C}_6\text{H}_3 \underset{\text{NMe}}{\overset{\text{CH.CH:CH}_2}{\diamondsuit}} \text{C:CH}_2$$
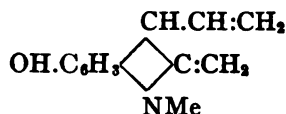
and (2) a phenolic base *physostigmol* to which the formula

$$\text{OH.C}_6\text{H}_3 \underset{\text{NMe}}{\overset{\text{C.CH:CH}_2}{\diamondsuit}} \text{CH}$$
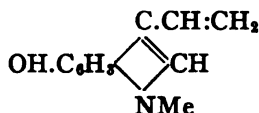
is assigned. It is optically inactive and gives a *picrate* crystallising in red needles m. p. 161–162°. Ethylamine and dimethylamine were also obtained.

Physostigmine forms an aurichloride, B, 2HAuCl_4, yellow leaflets, m. p. 163–165° and a platinichloride, B, H_2PtCl_6, orange yellow needles, m. p. 180° (decomp.).

Alkaloids of Ergot.

Recent unpublished observations have thrown much doubt on the statement on page 20, Vol. VII, that ergotoxine contains a carboxyl group. The figures 4 and 5 do not represent salts of ergotoxine ethyl ester, but of ergotoxine itself. Thus the phosphate at least appears to occur in two crystalline forms.

The Alkaloids of Jaborandi.

The leaves of *Pilocarpus microphyllus* contain the alkaloids pilocarpine and isopilocarpine, but no pilocarpidine.[1] After removing the first two mentioned bases as completely as possible there still remains a residue which consists of a mixture of bases. From this mixture a new alkaloid *pilosine* (carpiline) has recently been isolated by Pyman[2] and almost simultaneously by Léger and Roques.[3]

The isolation of pilosine was carried out by Pyman in the following manner. The residual syrup (after complete extraction of pilocarpine and isopilocarpine) from several tons of leaves was diluted with water, and the bases precipitated by addition of ammonia. There was first precipitated a dark coloured oil and later a lighter coloured oil. These fractions were redissolved in dilute acid, and again fractionally precipitated by ammonia when the impurities became concentrated in the earlier dark coloured fractions. The latter fractions of the sparingly soluble oily precipitate when dissolved in a little alcohol readily deposited crystals of the new base pilosine. Purification was effected by recrystallisation from alcohol.

Léger and Roques proceed as follows: Pilocarpine and isopilocarpine were removed as completely as possible as nitrates or hydrochlorides. The residual bases were precipitated by ammonia and the first fractions· of the

[1] *Jowett, Trans.*, 1900, **77**, 473.
[2] *Trans.*, 1912, **101**, 2260.
[3] *Compt. Rend.*, 1912, **155**, 1088.

precipitate purified first by crystallisation from absolute alcohol and later from 90% alcohol.

According to Pyman the amount of pilosine present in the leaves of *Pilocarpus microphyllus* amounts to about 0.007%. No other alkaloid is present in amount greater than 0.003%. The absence of pilocarpidine was also confirmed.

Pilosine, $C_{16}H_{18}O_2N_2$, crystallises from alcohol in colourless plates, m. p. 187° (corr.) (Pyman); in prisms, m. p. 184°–185° (Léger Roques). It is sparingly soluble in cold water, chloroform, ether, ethyl acetate and benzene, but fairly readily soluble in hot water or alcohol. The base is dextrorotatory. In chloroform it has $[\alpha]_D + 40.2°$, in alcohol $[\alpha]_D + 39.9°$. It is a feeble monacid base and does not readily form crystalline salts, although several have been prepared and described. Like pilocarpine it contains a lactone group and hence dissolves in hot caustic alkalis. It gives no colouration with sodium diazobenzene-*p*-sulphonate.

Pilosine *sulphate*, $(B)_2,H_2SO_4$, crystallises from alcohol in plates, m. p. 194°–195° (corr.). The *hydrogen tartrate* $B,C_4H_6O_6$, separates from alcohol as an oil which slowly crystallises and melts at 135°–136° (corr.). The *aurichloride*, $B,HAuCl_4$, crystallises from glacial acetic acid in golden-yellow wedge-shaped plates, m. p. 143°–144°. A crystalline *hydrochloride*, B,HCl, a *platinichloride*, $B,H_2PtCl_6,5H_2O$, and a *methiodide*, B,CH_3I, crystallising in pale yellow prisms have also been described.

On treatment with acetic anhydride (best by boiling with a mixture of equal parts of acetic anhydride and glacial acetic acid) pilosine loses the elements of water and a new base, *anhydro-pilosine*, which yields well crystalline salts, is produced.

Anhydro-pilosine, $C_{16}H_{16}O_2N_2$, crystallises from ethyl acetate in colourless rods, m. p. 133°–134° (corr.). It is sparingly soluble in cold, easily soluble in hot water, and in the usual organic solvents with the exception of ether. The base is unsaturated, contains a lactone group, and is optically active. In alcoholic solution it has $[\alpha]_D + 66.2°$.

The *sulphate*, B_2,H_2SO_4, prisms m. p. 174° (corr.), the *nitrate*, B,HNO_3, prisms m. p. 153°–154° (corr.) and *hydrogen oxalate*, $B,C_2H_2O_4$, needles m. p. 153°–154° (corr.) have been described. *Pilosinine*, $C_9H_{14}O_2N_2$, is obtained when pilosine is distilled with 20% aqueous potassium hydroxide solution, benzaldehyde being produced at the same time. In order to isolate the base the alkaline distillation residue is rendered acid and boiled for a few minutes, to reform the lactone group. The solution is then made alkaline with ammonia, and the base is extracted with chloroform and converted into the crystalline nitrate. From a solution of the nitrate the pure base is obtained by extraction with chloroform after addition of ammonia.

Pilosinine crystallises from ethyl acetate in plates, m. p. 78°–79° (corr.). It is somewhat deliquescent, readily soluble in water, alcohol, chloroform, and hot ethyl acetate, sparingly soluble in dry ether. The base may be

distilled at about 300° (35 mm.). It is optically active; in freshly prepared aqueous solution it has $[\alpha]_D + 41.2°$. As in the case of pilocarpine, however, the rotation rapidly falls on keeping, presumably owing to the conversion of the lactone into a hydroxy-acid.

The *nitrate*, B,HNO₃, prisms m. p. 165°–167° (corr.) and the *hydrochloride*, B,HCl, prismatic needles m. p. 218°–219° (corr.) are readily obtained.

Both anhydro-pilosine and pilosinine are also obtained by heating pilosine with water to 140° for 10 hours.[1]

Constitution of Pilosine.—The chemical and physiological properties of pilosinine very closely resemble those of pilocarpine. Moreover pilosine, the parent base of pilosinine, occurs in the same plant as pilocarpine and isopilocarpine, and it therefore appears highly probable that pilosinine is a glyoxaline derivative closely analogous to pilocarpine. Bearing these facts in mind, the constitution of pilosinine may be satisfactorily represented by the formula

$$CH_2\text{—}CH\text{—}CH_2\text{—}C\text{—}NMe$$
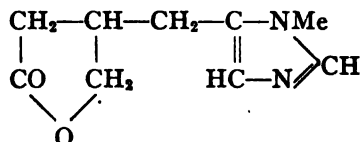
Further, from the facts that on distillation with strong aqueous sodium hydroxide pilosine yields benzaldehyde and pilosinine, and that by removal of the elements of water from pilosine the unsaturated base anhydropilosine is produced, Pyman concludes that pilosine must have the constitution denoted by the formula (I)

$$C_6H_5.CHOH.CH\text{—}CH\text{—}CH_2\text{—}C\text{—}NMe \quad \text{I.}$$

$$C_6H_5.CH{:}C\text{—}CH\text{—}CH_2\text{—}C\text{—}NME$$

II.

$$CH_2\text{—}CH\text{—}CH_2\text{—}C\text{—}NMe$$

$$+C_6H_5.CHO$$

III.
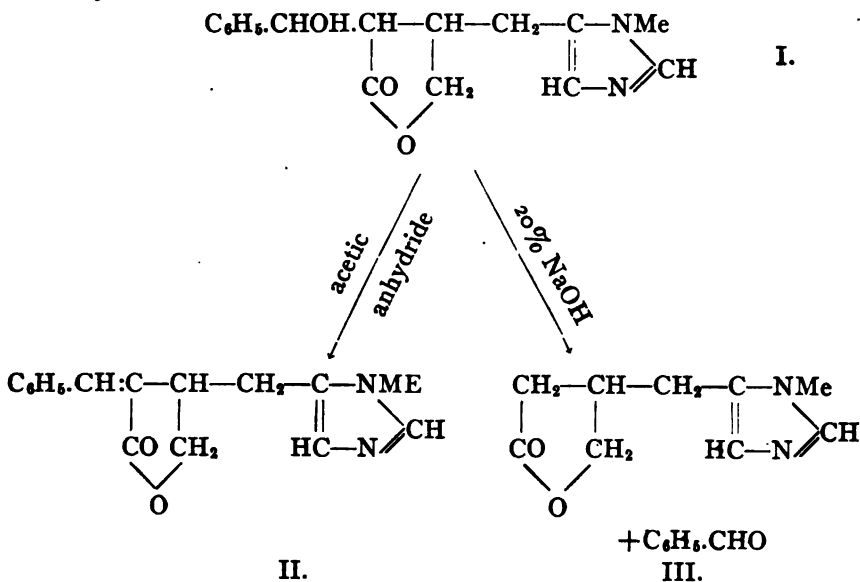
[1] Léger and Roques, *Compt. rend.*, 1913, 156, 1687.

The formation of anhydro-pilosine (II) and of pilosinine (III) is satisfactorily represented as shown. The decomposition of pilosine into benzaldehyde and pilosinine by alkali is supported by the fact that pilosine is, on the above assumption, the lactone of a β-hydroxy-acid which under certain circumstances is known to be decomposed by alkali forming an aldehyde and an acid.

Physiological Action.—According to Laidlaw (quoted by Pyman), pilosine and anhydro-pilosine have a very feeble pilocarpine-like action, in that they produce (in 20 mg. doses) a weak inhibition of the cat's heart. Pilosinine, however, has a more marked, though mild, pilocarpine action inhibiting the heart of cats and frogs and producing some salivary secretion.

Solanine Alkaloids.

A recent investigation of the constituents of *Solanum angustifolium* by Tutin and Clewer[1] has resulted in the isolation of a new solanine base, a gluco-alkaloid to which the authors have given the name solangustine. *Solanum angustifolium* occurs in several countries in South America, where it is known as "Duraznillo Blanco" and is employed as a febrifuge, chiefly in the treatment of enteric fever. The material employed was obtained from Lima (Peru) and was botanically identified.

For the isolation of the alkaloid the following procedure was adopted: The completely dried material (leaves, twigs, and flowers) was completely extracted with hot alcohol. The residue after removal of the solvent was mixed with water and distilled with steam to remove the last traces of alcohol. The residual aqueous liquid was removed from the resin and the liquid extracted with amyl alcohol. The numerous extracts were combined, washed with water, and concentrated. The residue was then distilled with steam to remove amyl alcohol completely, and the dark-coloured aqueous liquid extracted several times with ether to remove impurities. The solution was then treated with about 5% of its weight of sulphuric acid, warmed gently for 15 minutes, cooled and the precipitated sulphate filtered off. For further purification it was well washed with boiling alcohol, and then digested with slightly diluted acetic acid when, without dissolving to any appreciable extent, it became crystalline. From the sulphate the free base was obtained by treating with warm sodium carbonate solution and extracting with warm amyl alcohol. The washed amyl alcohol solution on concentration deposited the free base as hard pale yellow aggregates of crystals. The amount present was about 0.065% of the dried material.

Solangustine, $C_{33}H_{53}O_7N,H_2O$, darkens at 225° and melts at 235° (decomp.). It contains 1 H_2O and when anhydrous rapidly reabsorbs water from the air. The base is characterised by extreme insolubility in nearly all solvents and by the insolubility of its salts. Solangustine dissolves fairly readily in pyridine, but scarcely at all in any other solvent.

[1] *Trans.*, 1914, 105, 564.

It contains no methoxyl group, and does not yield a crystalline acetyl derivative. The only crystalline salt obtained was the sulphate $B_2,H_2SO_4,$-$3H_2O$. On shaking an amyl alcohol solution of the base with dilute sulphuric acid it separates as small acicular crystals which do not melt or decompose up to 325°. It crystallises with $3H_2O$ and when anhydrous is very hygroscopic.

On hydrolysis solangustine yields dextrose and an amorphous base *solangustidine*, $C_{27}H_{43}O_2N$, which forms crystalline salts. The hydrolysis is thus represented:

$$C_{33}H_{53}O_7N + H_2O = C_{27}H_{43}O_2N + C_6H_{12}O_6$$
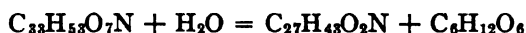
Solangustidine, $C_{27}H_{43}O_2N$, is insoluble in water, soluble in dilute alcohol, from which it separates in amorphous granules. On treatment with acetic anhydride, *acetylsolangustidine*, $C_{27}H_{42}O_2NAc$, crystallising from ethyl acetate in needles, m. p. 256°, is obtained. This compound is remarkable for its stability towards alkalis, being recovered unchanged after several hours' heating with alcoholic potassium hydroxide.

Solangustidine forms well crystalline salts which are practically insoluble in water.

The *hydrochloride*, B,HCl, crystallises from alcohol (containing a little alcoholic HCl) in lustrous plates, m. p. above 325°. It is sparingly soluble in amyl alcohol or hot ethyl alcohol.

The *hydrobromide*, B,HBr forms plates, m. p. 320° (decomp.). It is rather more readily soluble in alcohol than the hydrochloride.

The *nitrate*, B,HNO₃, is fairly soluble in hot dilute nitric acid. It darkens at 260° and decomposes at 290°.

The *sulphate*, B_2,H_2SO_4, was obtained crystalline by boiling the amorphous precipitate, obtained by treating an alcoholic solution of solangustidine with dilute sulphuric acid, for some time. Colourless leaflets, m. p. above 330°. Sparingly soluble in water.

The *picrate*, $B,C_6H_3O_7N_3$, forms yellow needles, m. p. 250° (decomp.).

The Alkaloid of the Common Broom.

Sparteine, $C_{15}H_{26}N_2$.—A number of salts of this base have been described by Corriez.[1] Among them *sparteine perbromide*, $C_{15}H_{26}H_2,2HBr,Br_2,H_2O$, is obtained by the action of bromine on sparteine, in fuming hydrobromic acid solution. The salt forms small yellow crystals, m. p. 193°, and its formation constitutes a delicate *test* for sparteine, since it is stated to be produced in a dilution of one part in ten thousand.

Alkaloids of Ipecacuanha.

The interest attached to these alkaloids has recently been greatly increased by the discovery by Vedder[2] of the intensely toxic action of emetine

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[1] *Bull. Sc. Pharmacol.*, 1912, 19, 468–480 and 533–540.
[2] *Journ. Trop. Med.*, 1911, 14, 149 (abstr.).

on amœbæ, and its therapeutic application by Rogers[1] to the treatment of amœbic dysentery (hypodermic injection of the hydrochloride). As a result several papers dealing mainly with the constitution and characterisation of these alkaloids have appeared during the last 2 years.

Of these, perhaps the most important publication is that of Carr and Pyman.[2] These authors have fully investigated the subject of the ipecacuanha alkaloids with the result that much of the older work must now be discredited. In particular they have succeeded in establishing the relationship existing between the three alkaloids emetine, cephaeline, and psychotrine and have thrown some light on their constitution.

From the results of a very large number of analyses of the pure bases and of their respective salts they reject the older formulæ assigned by various workers to these alkaloids and adopt the following: emetine, $C_{29}H_{40}O_4N_2$; cephaeline, $C_{28}H_{38}O_4N_2$; psychotrine, $C_{28}H_{36}O_4N_2$. Emetine is, therefore, cephaeline + CH_2, and cephaeline is psychotrine + H_2. This relationship was indeed confirmed experimentally, since psychotrine on reduction gave two isomeric dihydro derivatives one of which was identical with cephaeline, while cephaeline in turn on methylation under suitable conditions gave, among other products, a base which was identified as emetine.

The material used in their investigation was Brazilian ipecacuanha root which contained 2.7% of total alkaloids. From this they obtained 1.35% of pure emetine, 0.25% of cephaeline, and a small amount of psychotrine. The method employed to isolate the alkaloids was that originally described by Paul and Cownley.[3]

A recent paper by Hesse[4] describes two new alkaloids, *ipecamine*, $C_{28}H_{36}O_4N_2$, and *hydro-ipecamine*, $C_{28}H_{38}O_4N_2$, in addition to those already mentioned, but these are amorphous and are at present not sufficiently well characterised to warrant unhesitating acceptance. To emetine he assigns the old formula $C_{30}H_{40}O_5N_2$ originally proposed by Kunz Krause, which was arrived at from analyses of what was undoubtedly a mixture of emetine and cephaeline.

The following description of the alkaloids emetine, cephaeline, and psychotrine is based on Carr and Pyman's paper.

Emetine, $C_{29}H_{40}O_4N_2$, m. p. 74° (corr.), has not so far been crystallised. It is obtained as a white amorphous powder by treatment of solutions of its salts with dilute alkalis, or as a varnish by the spontaneous evaporation of its solutions in organic solvents. It is readily soluble in methyl, ethyl, or amyl alcohol, in ethyl acetate, ether, acetone, or chloroform; less soluble in benzene or light petroleum. Molecular weight determinations and titration experiments support the view that the base contains two

[1] *Brit. Med. Journ.*, 1912, i, 1424.
[2] *Trans.*, 1914, 105, 1591.
[3] Vol. VII, p. 38.
[4] *Annalen*, 1914, 405, 1.

nitrogen atoms in the molecule, evidence of formation of basic salts having been obtained.

Emetine is lævorotatory. In chloroform solution it has $[\alpha]_D - 49.7°$, and this is independent of the concentration. In dissociating solvents, however, its rotation varies considerably. The following salts were characterised:

The *hydrochloride*, $B,2HCl,7H_2O$, woolly needles from water. Crystallised from methyl alcohol the salt contains only $3\frac{1}{2}$ H_2O. The anhydrous salt sinters at 235° and decomposes at 255° (corr.). A saturated aqueous solution at 18° contains 13.1 grm. of hydrated salt in 100 c.c. of solution.

The *hydrobromide*, $B,2HBr,4H_2O$, long slender needles from water. Sinters at 245° and melts from 250°–265° (corr.). Sparingly soluble in cold, readily soluble in hot water. 100 c.c. of a saturated solution at 18° contains 1.9 grm. of hydrated salt.

The *hydriodide*, $B,2HI,3H_2O$, crystallises from alcohol in needles and is sparingly soluble in water. It sinters from 230° and melts at 235°–238° (corr.).

The *nitrate*, $B,2HNO_3,3H_2O$, crystallises from water or alcohol in fine needles. It sinters from 188° and gradually melts up to 245° (corr.). It is sparingly soluble in water.

The *sulphate*, $B,H_2SO_4,7H_2O$, forms white woolly needles and is very soluble in water. It sinters at 205° and finally melts at 245° (corr.). The *acetate* and *platinichloride* were not obtained crystalline.

Benzoylemetine, $C_{29}H_{39}O_4N_2OC.C_6H_5$, crystallises from alcohol in needles, m. p. 185°–186° (corr.). Keller's "benzoylemetine" appears to have been the benzoate of benzoylemetine.

Cephaeline, $C_{28}H_{38}O_4N_2$.—The pure base is best obtained by regenerating it from a pure salt (hydrochloride or hydrobromide) and subsequently recrystallising from ether. It forms colourless needles which, dried in the air, sinter at 106° and melt at 115°–116° (corr.). After drying at 100° it melts gradually from 120°–130°. It is readily soluble in chloroform, alcohol or acetone, sparingly so in ether or light petroleum. The base is apparently somewhat unstable. Its solutions darken on keeping and the base itself becomes coloured on drying at 100°. Cephaeline is lævorotatory. In chloroform solution it has $[\alpha]_D - 43.4°$. The hydrochloride and hydrobromide were alone obtained crystalline.

The *hydrochloride*, $B,2HCl,7H_2O$, crystallises from dilute hydrochloric acid in stout prisms or from water as fine powdery crystals. The salt has no sharp melting point, but sinters at 245° and finally melts at 270° (corr.). A saturated aqueous solution at 18° contains 26.5 grm. of hydrated salt per 100 c.c.

An *acid hydrochloride*, $B,5HCl$, separates from strongly acid solutions in fine needles, m. p. 84°–86°.

The *hydrobromide*, $B,2HBr,7H_2O$, crystallises from dilute hydrobromic acid. It sinters at 266° and melts up to 293°. 100 c.c. of a saturated aqueous solution at 18° contains 5.4 grm. of anhydrous salt.

Psychotrine, $C_{28}H_{36}O_4N_2$, was first obtained by Paul and Cownley as yellow prisms, m. p. 138°. It crystallises from wet acetone or alcohol in large yellow prisms (with a blue fluorescence) which contain $4H_2O$. After drying at 100° it sinters at 120°, becomes transparent at 124°–126° (corr.) and melts at 138°. It is sparingly soluble·in water, benzene, petroleum, or ether, more readily soluble in acetone, alcohol or chloroform. Its solutions in alkalis or strong acids are colourless. In concentrated sulphuric acid on addition of a trace of nitric acid it gives a sherry colour. With excess of Fröhde's reagent it yields a pale green solution.

The *sulphate*, $B,H_2SO_4,3H_2O$, crystallises from water in faintly yellow scales. The anhydrous salt sinters at 207° and melts at 214°–217° (corr.).

The *nitrate*, $B,2HNO_3,H_2O$, crystallises from water in silky needles, sparingly soluble in cold water. The anhydrous salt sinters from 165° and melts at 184°–187° (corr.).

The *hydriodide*, $B,2HI$, crystallises from a slight excess of dilute hydriodic acid as sulphur-coloured microscopic needles which sinter from 200° and melt and decompose at 220° (corr.).

Constitution of Ipecacuanha Alkaloids.—As already mentioned, the relationship existing between emetine, cephaeline and psychotrine has been established by Carr and Pyman. Of the ultimate constitution of these bases little is known. It has been found, however,[1] that on oxidation with potassium permanganate emetine gives rise to 6:7-dimethylisoquinoline-1-carboxylic acid, and also to *m*-hemipinic acid and its imide. The alkaloids thus belong to the class of isoquinoline bases. Oxidation of emetine and of cephaeline with ferric chloride also gave rise to new bases which are of considerable interest from the point of view of the constitution of these alkaloids.

[1] Carr and Pyman, *Proc. Chem. Soc.*, 1913, **29**, 226. See also Windaus and Hermanns, *Ber.*, 1914, **47**, 1470.

GLUCOSIDES.

By E. FRANKLAND ARMSTRONG.

Synthetic Glucosides.—A number of glucosides of the chalkones, of which two—naringenin and hesperitin—occur naturally in plants, have been synthesised by Bargellini.[1] Helicin was condensed with hydroxy-derivatives of acetophenone in alcoholic solution in presence of 40% sodium hydroxide or by heating in presence of piperidine.

Synthetic glucosides of the purine bases are described by Fischer and Helferich[2] including those of theophylline, theobromine, adenine and hypoxanthine. By combining these with phosphoric acid, products resembling the nucleotides have been obtained.

Cyanogenetic Glucosides (Compare Vol. VII, p. 101).—The cyanogenetic plants of New South Wales have been investigated by Petrie[3] using sodium picrate paper as an indicator of the presence of hydrogen cyanide. Of 60 species stated to contain such glucosides 20 are grasses.

Phytosterolins.—A number of glucosides of phytosterols have been isolated from plants of which sitosterol, $C_{33}H_{56}O_6$, and stigmasterol, $C_{36}H_{60}O_6$, are types. They have been investigated by Power and Salway[4] who give a list of the known varieties. They are not affected by heating with aqueous or dilute alcoholic hydrogen chloride, but are hydrolysed by this acid when dissolved in warm amyl alcohol solution. They form crystalline tetra-acetyl- and tetrabenzoyl-derivatives and give the characteristic colour indication of the phytosterols when they are dissolved in acetic anhydride and chloroform and a drop of concentrated sulphuric acid is added. Sitosterol-d-glucoside melts at 270–300°; it can be prepared synthetically from sitosterol and acetobromoglucose.[5] Glucosides of cholesterol and of fatty alcohols have been synthesised in the same manner; it is probable that they will be found in plants.

Seeing that the tannins are acyl-derivatives of glucose of the type of penta-acetyl glucose it was to be expected that simpler acyl derivatives would be found to exist naturally. The first of these to be described is a crystalline bitter substance present in the leaves and stems of *Daviesia latifolia*.[6] This is a dibenzoyl derivative ($C_{25}H_{28}O_{12}$) of a disaccharide composed

[1] *Gazette*, 1914, 44, ii, 520.
[2] *Ber.*, 1914, 47, 210.
[3] *Chem. News*, 1914, 110, 126.
[4] *Trans.*, 1913, 103, 399.
[5] Salway, *Trans.*, 1913, 103, 1022–1029.
[6] Power and Salway, *Trans.*, 1914, 105, 767–778; 1062–1069.

of glucose and xylose, m. p. 147–148°. The glucoxylose has no reducing action on Fehling's solution and therefore is of the same type as saccharose.

Several species of the genus *Solanum* are reported to contain bases which are both alkaloids and glucosides. *Solangustine*, the gluco-alkaloid isolated from *Solanum angustifolium* by Tutin and Clewer[1] has the composition $C_{33}H_{55}O_7N$, m. p. 235° (decomp.). It is hydrolysed to glucose and solangustidine and is without physiological activity. The plant also contains quercetin, rutin and *l*-asparagine. Reference is given to similar gluco-alkaloids termed collectively solanines (see page 540).

Digitalis (Compare Vol. VII, p. 116).—According to Hirohashi[2] digitalis leaves gathered from different parts of the plant differ in their physiological effect, there being a diminution in the latter from the top downwards. The leaves are best collected before inflorescence. The flowers have a maximum of activity during budding; there is no difference in activity between red and white flowers. An infusion of the leaves can be evaporated without loss of any of its physiological effects.

Hatcher[3] states that digitalis of the first year's growth is probably as active as that of the second and the cultivated is as active as the wild-grown plant. When properly dried and stored, digitalis will keep indefinitely.

Martindale[4] claims that the following simple chemical method gives an approximate idea whether a tincture of digitalis is up to the physiological test requirements: 10 c.c. of the tincture are mixed with 10 c.c. of water and precipitated with 3 c.c. of 10% normal lead acetate solution, a little kieselguhr being added. After standing for 15 minutes the precipitate is filtered off and washed. The lead is removed from the filtrate by the addition of 2 c.c. of 10% sodium phosphate solution. The filtrate is evaporated to dryness after adding 0.2 grm. of calcium carbonate. The residue is mixed with sand and extracted five times with chloroform, using 10 c.c. on each occasion. The extract is evaporated and the residue extracted with warm water on the water-bath using 10 c.c. and 5 c.c. and again employing sand. The filtrate is evaporated to dryness and extracted three or four times with chloroform, 5 c.c. each time, the residue being mixed with sand and thoroughly triturated. The chloroform liquors are evaporated and the residue dissolved in 4 c.c. of glacial acetic acid. 0.1 c.c. of the acetic acid solution is mixed with 1 c.c. of "sulphuric ammonium molybdate solution" in a 5 × 1 cm. test-tube, and the depth of colour produced after 5 minutes is compared with a standard. The colouration indicates the content of combined "active water-soluble glucosides."

Reichard[5] describes a number of reactions of digitonin. A drop of cobalt nitrate solution is evaporated until a deep blue coloured residue is obtained; digitonin and a drop of glacial acetic acid are added and the mixture exposed

[1] *Trans.*, 1914, 105, 559.
[2] *Chem. and Drug.*, 1913, 82, 18.
[3] *Amer. J. Pharm.*, 1914, 86, 567.
[4] *Pharm. J.*, 1912, 35, 745, 778.
[5] *Pharm. Zentr.*, 1913, 54, 217.

to the air for 30 hours. A mass of red crystals is obtained whereas digitoxin only gives a green residue.

The digitonide of oxycholesterol, which occurs in animal fats in association with cholesterol, crystallises in rhombic plates, m. p. 215°.[1]

A new glucoside, **Gitonin** has been found in *Digitalinum germanicum*. It is separable from digitonin by taking advantage of its smaller solubility in 95% alcohol. It has the composition $C_{26}H_{44}O_4$ or $C_{26}H_{42}O_4$., m. p. 272°, $[\alpha]_D$ − 50.7° in pyridine. Acids hydrolyse it to galactose, a pentose and *gitogenin*, which has m. p. 272° and forms a *diacetate*, m. p. 243–244°.[2]

Digitoxin and Gitalin.[3]—When a solution of digitoxin, gitalin and anhydrogitalin in a mixture of equal volumes of chloroform and methyl alcohol is treated with ether, gitalin remains in solution and the two other constitutents are precipitated.

Strophanthin.—According to Lampart and Müller[4] who have compared a number of methods, the most satisfactory way of determining Strophanthin in strophanthus seeds or tincture, is an extension of that of Cæsar and Loretz described in Vol. VII, p. 122. The full details are as follows:

"7.0 grm. of the finely crushed seeds are boiled in a reflux apparatus for an hour with 70.0 grm. of absolute alcohol. When cold, the whole is made up to the original weight with absolute alcohol, and 50.5 grm. filtered into a porcelain basin. The alcohol is evaporated and the residue washed with light petroleum which is poured through a filter. The insoluble residues in the filter and basin are boiled with 5–8 grm. of water, treated with 5 drops of lead acetate solution and about 0.2 grm. of kieselguhr, well mixed and filtered into a 100 c.c. flask. The insoluble portion is washed till the runnings no longer have a bitter taste. The filtrate is treated with 5 drops of hydrochloric acid and boiled gently for 2 hours, the volume being kept between 10–20 c.c. by the addition of distilled water. When cold, the liquid is extracted twice with 10 c.c. of chloroform, which is filtered into a tared flask. The aqueous portion is again boiled for half an hour, cooled and extracted three times with 10 c.c. of chloroform. If the aqueous portion after warming still tastes bitter, the boiling and extraction with chloroform are repeated. The chloroform is distilled off, the residue dried in a desiccator and then weighed. It consists of strophanthidin, 1 part of which corresponds to 2.187 parts of pure strophanthin. For the tincture 51 grm. (equivalent to 5.0 grm. of the seeds) are heated on the water-bath to remove the alcohol, the residue is taken up with 20 grm. of hot water, treated with 15 drops of lead acetate solution and 0.2 grm. of kieselguhr. It is then treated by the method described above for the seeds."

The new method gives higher percentages than any of the others.

All varieties of the drug contain varying proportions up to 0.2% of the hæmolytic saponin, strophanthic acid.[5] This forms precipitates with salts

[1] Lifschutz and Grethe, *Ber.*, 1914, 47, 1453.
[2] Windaus and Schnechenburger, *Ber.*, 1913, 46, 2628.
[3] See Kraft, *Arch. Pharm.*, 1912, 250, 118, and Kiliani, *Ibid.*, 1913, 251, 562.
[4] *Arch. Pharm.*, 1913, 251, 609.
[5] Sieburg, *Ber. Pharm. Ges.*, 1913, 23, 278.

of heavy metals; it is hydrolysed to glucose and strophanthigenin. A list of colour reactions is given (see also Chem. Soc. Abstracts, 1913, i, 640) which distinguish strophanthic acid from strophanthin.

Sarsaparilla.—Sarsaparilla root has been fully investigated by Power and Salway[1] who worked with the grey Jamaica root of the British Pharmacopœia (*Smilax ornata*). The following compounds were obtained from the alcoholic extract:

(1) *Sarsasaponin*, a crystalline glucoside—$C_{44}H_{76}O_{20}$ (m. p. 248°, $[\alpha]_D$ − 48.5°) yielding glucose and sarsasapogenin on hydrolysis.

(2) *Sitosterol-d-glucoside* (a phytosterolin)—$C_{33}H_{56}O_6$ (m. p. 280–285°).

(3) *Sitosterol*, $C_{27}H_{46}O$ (m. p. 135–136°, $[\alpha]_D$ − 27.3°).

(4) *Stigmasterol*, $C_{30}H_{50}O$ which is identified by its tetrabromoacetyl derivative, $C_{30}H_{49}OBr_4.COCH_3$ (m. p. 208°).

(5) *Sarsapic Acid*—a new crystalline dicarboxylic acid (m. p. 305°), $C_4H_2O_2(CO_2H)_2$.

(6) *Glucose.*

(7) *Fatty Acids*—viz., palmitic, stearic, behenic, oleic and linoleic acids.

(8) *Cetyl-d-glucoside.*

(9) Potassium nitrate.

The root contains a small quantity of an enzyme of the emulsin type.

Inasmuch as only one definite saponin glucoside is present, it is considered that the parillin of earlier investigators consisted of a mixture of sarsasaponin and a phytosterolin. Commercial smilacin represents a relatively small proportion of sarsasaponin with indefinite amorphous products.

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[1] *Trans.*, 1914, 105, 201–209.

NON-GLUCOSIDAL BITTER
PRINCIPLES.

By G. C. JONES, F. I. C.

Aloes.

Detection of Aloes in Extracts of Drugs Containing Hydroxy-methyl-anthraquinones (cf. Vol. VII, pp. 149–150).—The following method will detect as little as 0.2 grm. of aloes extract in 5 grm. of a mixture of extracts of rhubarb, frangula and cascara sagrada.[1] The alcoholic extract is evaporated to expel the alcohol, the residue taken up in water and the liquid filtered. The filtrate (100 c.c.) is heated for 30 minutes on the water-bath with 5 c.c. of 10% sulphuric acid, the sulphuric acid precipitated with the exact amount ·of barium hydroxide solution, and the filtrate from the barium precipitate concentrated to 100 c.c. and clarified with lead acetate solution, care being taken to avoid a large excess. Of the solution, 10 c.c. is filtered, freed from lead by means of sodium sulphate solution or dilute sulphuric acid and divided into two portions. One of these is shaken with 5 c.c. of benzene and the benzene extract shaken with dilute ammonia. If the hydroxymethylanthraquinones have been completely precipitated, the aqueous layer should not show more than a light rose colour, whilst a yellow colouration of the benzene layer indicates aloes. If, however, the aqueous layer is distinctly red, the bulk of the original solution must again be treated with lead acetate, after which 10 c.c. is filtered, freed from lead, divided into two portions, and one of these portions tested with benzene and ammonia as before. When the precipitation of the hydroxymethylanthraquinones is shown to be complete, the other portion of the filtrate is treated with an excess of bromine water, which, in presence of aloin, gives an immediate flocculent precipitate. The main solution is now freed from lead and 10 c.c. portions of the filtrate are tested as follows. One portion is gently heated and shaken with 2–3 grm. of borax (cf. Vol. VII, p. 149) and allowed to stand for 15 minutes, when, in the presence of aloes, a green fluorescence appears. Another 10 c.c. portion is heated with 1 drop of copper sulphate solution and 1 drop of hydrogen peroxide solution (cf. Klunge's test and Léger's test, Vol. VII, pp. 144 and 149–150). In the presence of aloes, a red colouration is obtained which becomes intensified on standing, whilst samples free from aloes become orange-red and do not alter on standing.

[1] G. Mossler, *Pharm. Post.*, 1913, 46, 313, 325.

Identification of the Different Hydroxymethylanthraquinone Drugs in Admixture with Aloes.—The alcoholic extract is brought to a strength of 50% of alcohol and filtered. The filtrate is boiled for 30 minutes beneath a reflux condenser with about 5% of sulphuric acid, cooled and filtered, and the alcohol expelled from the filtrate by repeated evaporation with water. The hydroxymethylanthraquinones are precipitated, whilst aloin remains in solution. The precipitate is washed free from acid, dried, boiled with benzene, and the benzene extract shaken with 10% sodium carbonate solution and then with dilute sodium hydroxide solution. The two alkaline extracts are separately acidified with hydrochloric acid, each shaken with 20 c.c. of benzene, and 5 c.c. of each benzene extract is evaporated. The residues are heated with 3–4 drops of acetic acid, and the solutions transferred to glass slips and examined, after 30 minutes, in polarised light. The crystals thus obtained from rhubarb, cascara sagrada, senna, etc., show pronounced differences, as is also the case with crystals obtained by sublimation.

Artemisia Bitters.

Estimation of Santonin in Wormseed.—The following method, due to Fromme, has recently been favourably reported on by C. E. Caspari.[1] The finely powdered wormseed (13 grm.) is macerated with occasional shaking with 130 grm. of chloroform. 102.5 grm. of the liquid ($=$ 10 grm. of the drug) is drawn off and evaporated until the residue weighs only 7 to 8 grm. This residue is mixed with 100 c.c. of 5% barium hydroxide solution and heated on the water-bath until the odour of chloroform has disappeared. The liquid is filtered through a wet filter and the insoluble matter washed twice with 10 c.c. of hot water. The filtrate and washings are acidified with 5 c.c. of 25% hydrochloric acid, heated on the water-bath for a few minutes, cooled and shaken out with 20, 15 and 15 c.c. of chloroform. The extracts are filtered and evaporated to dryness. The residue is dissolved in 7.5 grm. of absolute alcohol and 42.5 grm. of hot distilled water are then added. The milky liquid is filtered immediately into a tared flask and the filter washed twice with 10 grm. of 15% alcohol. After 24 hours, the liquid is filtered through a tared filter, and the flask and filter washed twice with 10 grm. of the dilute alcohol. The flask and filter are finally dried at 100° C. to constant weight, 0.04 grm. being added to the weight of santonin found.

Hops.

Since the proofs of Vol. VII were passed, Power, Tutin and Rogerson[2] have published an elaborate paper on the Constituents of Hops, and Chapman[3] a short note on the Nitrogenous Constituents of Hops. If the descriptive

[1] *Amer. Pharm. Assoc.*, 1914, 634.
[2] *Trans.*, 1913, 103, 1267.
[3] *Proc. Chem. Soc.*, 1913, 29, 182.

matter, which preceded the instructions given in Vol. VII (pp. 164-175) for the commercial analysis of hops, purported to be exhaustive, it would be necessary now to refer in detail to the above-mentioned papers. As, however, the plan of this work is to exclude descriptive matter except so far as this is necessary to explain the principles on which commercial methods of analysis are based, Chapman's paper, although adding materially to our knowledge, and although the work of an author who has made a special study of hops from both the chemical and technological points of view, needs only to be cited here, pending his promised investigation of the technological significance of the constituents he has isolated.

The question of the treatment that should be accorded to the work of Power and his collaborators presents more difficulty, but the writer feels that a general discussion of their results may well be deferred. Since, however, these authors express the opinion that their results undermine the whole principle on which the current methods for the commercial analysis of hops depend, this claim is criticised at some length in a subsequent paragraph dealing with the Estimation of Soft and Hard Resins. Power and his collaborators have derived from hops a great number of compounds, including minute quantities of two new crystalline, phenolic substances, one of which is bitter, and notable quantities of fatty acids. It is on the latter ground, mainly, that they attack current methods of analysis.

Yet there is no evidence in their paper that hops contain fatty acids as such, or many other of the compounds that they isolated. That fatty esters would be present in an extract of ground Kentish hops would have been expected by anyone conversant with the fact that the seeds of the hop contain upwards of 25% of a fatty oil (Vol. VII, p. 177), and that the hops employed by them were well seeded may be inferred from the fact that the ground sample yielded no less than 21.8% of matters soluble in petroleum ether. The isolation of fatty acids was preceded by saponification with alcoholic potash and it has been known for 10 years that one of the best characterised constituents of the "soft resin" (the so-called α-acid) yields valeric acid on such treatment, which would also saponify the fatty oil derived from the seeds and not improbably break down other constituents of the resin. It is as remarkable as it is unfortunate that chemists of the unquestioned attainments of Power and his collaborators should have studied—as they appear to have studied—the literature of the subject, without appreciating the extraordinary instability of many of the constituents of the hop, and that they should have embarked on this elaborate research without due regard to this fact. Even before their treatment of the petroleum extract with alcoholic potash, they had probably brought about profound changes in its composition, for they subjected it twice to distillation in a current of steam, once for 5 hours. As Chapman pointed out in the discussion of their paper,[1] the fact that certain of the petroleum-

[1] *Proc. Chem. Soc.*, 1913, 29, 180.

soluble and preservative constituents might be converted, by simply boiling with water, into products insoluble in petroleum and possessed of little or no preservative properties, was not only well known, but was one of the facts that a well-planned study of the constituents of hops would have sought to explain. Enough has been said to show that the painstaking work of Power and his collaborators was not planned in such a way as to throw light on this and cognate questions, and, important as some of their results may ultimately prove, one must agree with Chapman that their immediate practical value is small.

The recent work of Brown and Clubb[1] makes it necessary to correct certain statements in Vol. VII. Applying the method of Brown and Ward (Vol. VII, p. 179), they find that the relation between the antiseptic power of hops and their total content of soft resins is much less simple than was at one time supposed. That the relationship was one of strict proportionality was never held, as it has always been recognised that the soft resin was a mixture in variable proportions of at least four substances, whilst, so long ago as 1901, Barth announced that he had secured direct evidence that one of the constituents was more powerfully antiseptic than another. Nevertheless, there was a general impression, which found support in the practical experience of brewers, that the total content of soft resins was a very fair measure of the antiseptic properties of hops. As evidence of this may be cited the facts that the determination of soft resins was constantly asked for by brewers and carried out by chemists, whereas the method of Brown and Ward received little attention until the appearance of the further paper by Brown and Clubb.

These authors have shown that two samples of hops of equal resin content may differ in antiseptic power in the rato of 2 : 1 when tested by the direct method of Brown and Ward, and that two samples of hops of equal antiseptic power when tested by that method may differ in soft resin content in the ratio of 2 : 1. This is the approximate order of magnitude of the maximum divergencies observed and is of course serious. At the same time they have shown that the antiseptic power of the α-acid is about four times as great as that of the β-acid. Barth stated that the β-acid was the more powerful, but he gave no figures, and in 1901 no precise method of measurement was available, so that the result of Brown and Clubb must stand for the moment, although they are careful to say that they were unable to obtain either acid in a crystalline condition. In view of the fact that the crystalline acids are so insoluble that they could hardly function as such in the Brown and Ward process any more than in the brewer's copper, but would first go over into the corresponding resins, the failure of Brown and Clubb to obtain them in crystalline form may be unimportant.

Since it is known that the more powerfully antiseptic α-resin may con-

stitute anything from 5% to nearly 50% of the total soft resin,[1] it seems probable that a constant relationship may be found to exist between the antiseptic power of hops and 4A + B, where A and B represent the percentages of α- and β-resins respectively. Brown and Clubb have published no data bearing on this point, but it appears deserving of investigation, as the wide variation in the ratio of α-resin to total soft resin and the great superiority of the α-resin as an antiseptic make it possible for two hops of equal soft resin content to differ even more widely in antiseptic power than any pair of examples cited by Brown and Clubb and may afford a complete explanation of their results above referred to.

Other results of Brown and Clubb are less easy of explanation. Chapman cites them[2] as having shown that a period of extraction quite insufficient for the solution or removal of the resins will suffice to extract the substance or substances on which the preservative properties of hops depend. A careful perusal of their paper, however, discloses the fact, not unexpected by one familiar with the praiseworthy care not to overstate his case that characterises all the utterances of A. J. Brown, that the authors do not claim to have proved quite so much as Chapman states. They say—and rightly say—that certain results of theirs do suggest such a conclusion as Chapman draws, but they are careful not to claim that their results amount to a proof, and the writer thinks this a convenient opportunity to point out that other explanations—less subversive of all our earlier views on hops— may possibly be found for their results. What they in fact found was that an aqueous extract of hops, prepared according to the method of Brown and Ward, did not contain more resin than corresponded to 23% of the soft resin in the original hops used. In another experiment, with hops of a different kind, it was found that when these were extracted as directed by Brown and Ward, then reextracted twice more in a similar manner, and the antiseptic powers of the successive extracts compared, these antiseptic powers stood in the ratio of 100 : 20 : 8. Combining these results—which may or may not be justified as the hops differed—one gets the suggestion that some constituent of the hop resin may have an antiseptic power 12 times as great as that of the others. Brown and Clubb, however, did not isolate any constituent possessed of such properties, and it is at least possible that their results may be explained without assuming the existence of such a substance. Their own results show that even 1 hour's extraction with water at 100° under their conditions leads to profound changes in the original soft resins, other than those extracted during the process. A portion of these soft resins is converted into hard resin, which would not confer any antiseptic properties on water subsequently boiled with the hops, and this transformation of soft resin into hard resin might be expected to continue during the second and third periods of extraction. Even the

[1] H. V. Tartar and B. Pilkington, *J. Ind. Eng. Chem.*, 1913, **5**, 478.
[2] *Analyst*, 1913, **38**, 599.

resins remaining soluble in petroleum after this treatment may have under-
gone a change involving a diminution in their antiseptic power. It is
recognised that this attempt to explain Brown and Clubb's results, without
resort to the hypothesis of some constituent of far higher antiseptic power
than is possessed by any yet isolated, implies the suggestion that an extract
prepared in accordance with the directions of Brown and Ward may have
only about half the antiseptic power of the hops from which it is prepared,
the antiseptic power of the unextracted soft resins, even if consisting wholly
of the less toxic β-resin, being largely destroyed by the process of extraction.
This, however, does not touch the principle of Brown and Ward's method
as a practical test of the brewing value of copper hops, since Brown and
Ward's extraction process is not very different from a brewery boil. It
also follows, if the writer's suggestion is correct, that only a portion—per-
haps less than half—of the preservative constituents of hops finds its way
into a brewer's wort, but this has long been suspected. What is not yet
known with certainty is whether the practical preservative power can be
expressed as a function of the content of α- and β-resins. That preservative
power is not proportional to $\alpha + \beta$ is amply proved, but that it may be
proportional to $k\alpha + \beta$, where k is a constant approximating 4, is suggested
as distinctly probable by the work of Brown and Clubb and that of others
read in the light of these authors' results.

Commercial Analysis of Hops.

Estimation of Soft and Hard Resins.—Some years ago, Lintner[1] and
Siller[2] stated that, unless hops were finely comminuted, petroleum ether
failed to extract the soft resins completely, and it has become customary
on the Continent to pass hops through a mincing machine[3] before proceeding
to the estimation of soft resins. With Continental hops, substantially free
from seeds, there is no objection to this fine grinding, but with seeded
British or American hops such treatment ruptures the seeds, liberating the
fatty oil contained in them (Vol. VII, p. 177) and cannot be resorted to
where the soft resins are finally to be estimated by a simple gravimetric
method such as that of Briant and Meacham (Vol. VII, p. 175). No objection
attaches to fine grinding, even with seeded hops, if the final estimation is
made by Lintner's volumetric method (Vol. VII, p. 177), which depends on
the acid function of the soft resins.

When the manuscript of Vol. VII was penned, however, Lintner's volu-
metric method was very little used in Britain, where the method of Briant
and Meacham was generally employed. For these reasons, among others, no
reference was made in Vol. VII to the then recent German proposals to resort
to fine grinding. Such procedure could not be applied to British hops

[1] *Chem. Zeit.*, 1908, 32, 1068.
[2] *Zeitsch. Nahr. Genussm.*, 1909, 18, 241.
[3] D. Neumann, *Wochenschr. f. Brauerei*, 1910, 27, 281.

without abandoning the analytical method in most general use, and at
that date there was no evidence that the results obtained by the method of
Briant and Meacham were liable to serious error.   Such evidence as ex-
isted that extraction with petroleum ether was incomplete, unless fine
grinding were resorted to, rested on experiments in which the period of
extraction was much shorter than the 24 hours directed by Briant and
Meacham.

On the Continent, there has always been a tendency to reduce the time
of extraction and, when following the directions for extraction given by
Lintner in the original description of his volumetric method (Vol. VII, p.
177), this appears to be permissible.   An objection to that method, that has
tended to restrict its use here, is the large volume of petroleum ether re-
quired (500 c.c.), but the use of a large volume of solvent maintained with
the hops at 50° C. does make it possible to reduce the time of extraction
materially below that required when a Soxhlet extractor is used.   A few
experiments by the writer confirmed Lintner's claim that 8 hours was suf-
ficient, but it is possible that with some hops extraction would be incomplete.
When using a Soxhlet extractor, the error attending a reduction of the
period of extraction varies according to the sample, but with some samples
is large.   Since 1908 or thereabouts, the inconvenience attending the use
of large quantities of solvent appears to have led German chemists to return
to the use of a Soxhlet extractor.   Siller (*loc. cit.*) makes mention of the use
of a Soxhlet apparatus and, since he extracted for only 10 hours, his dis-
covery that unground hops could not be completely extracted in this time
accords with British experience and does not appear to touch the principle of
Briant and Meacham's method.

More recently, however, this method has been seriously impugned by
Tartar and Bradley.[1]   These writers show a more intimate acquaintance
with the work of others than is usual among writers on hops, and it must
be assumed that the method they condemn as giving low results is the un-
amended method of Briant and Meacham, including the 24 hours' period of
extraction.   They say that the method of Briant and Meacham gives very
much lower results for soft resins, and correspondingly high ones for hard
resins, than does the latest form of Lintner's volumetric method or a new
gravimetric method now described by them.   In one case the latter methods
discovered 16% of soft resin, whereas the process of Briant and Meacham is
said to have extracted only 8%.   The results of Lintner's original method
are said to be in good agreement with those obtained by the method of
Briant and Meacham, but both methods are said to fail because, without
previous grinding, the whole of the soft resin cannot be extracted by petroleum
ether.

Tartar and Bradley (*loc. cit.*) have devised a new gravimetric method
which can be applied even to finely ground, seeded hops.   They claim, prob-

[1] *J. Ind. Eng. Chem.*, 1912, **4**, 209.

ably with justice, that their method is the most exact yet available, but it will not be described here as it is very tedious and as its authors themselves express the opinion that Lintner's modified volumetric method (described below) is the best method for commercial purposes, since it is rapid and yields results which seldom differ by more than a few tenths of 1% from those yielded by their own exact method.

Lintner's Modified Volumetric Method for the Estimation of Soft Resins differs from that described under his name in Vol. VII, p. 177 only in that the hops are first put through a mincing machine, the first portions being rejected, and are then extracted in a Soxhlet extractor for 8–10 hours with a minimum quantity of petroleum ether at the comparatively low temperature determined by this method of extraction, instead of at 50° C. in a flask with 300 c.c. of petroleum ether.   That on one and the same extract, prepared from uncrushed hops, Lintner's titration method gives substantially the same results as evaporation of the extract as directed by Briant and Meacham has long been known.   That Briant and Meacham's method cannot be applied to crushed hops is obvious, whereas Tartar and Bradley have shown conclusively that no such objection attaches to Lintner's titration method.   If Tartar and Bradley are also right in stating that the method of Briant and Meacham may give low results owing to incomplete extraction, the modified Lintner method stands out as the simplest method by which the soft resins can be estimated accurately.

The writer thinks it right to state that he has been unable to confirm Tartar and Bradley's observation that Briant and Meacham's method may lead to substantial error, and the continued use of that method in well-informed circles in Britain suggests that the explanation of Tartar and Bradley's results may possibly lie in some experimental detail hitherto not recognised as important.   On the other hand, the writer's own experiments to elucidate the point were few in number and a great deal of negative evidence would be necessary to outweigh the definite finding of Tartar and Bradley.   In view of thé facts that grinding makes it possible to complete the extraction in 8 hours instead of 24, without the use of inconvenient quantities of solvent, and that such grinding of British hops renders Briant and Meacham's method inaccurate, Lintner's modified method has now much to recommend it, even if further experiment should free Briant and Meacham's method from the aspersions cast on it by Tartar and Bradley.

A more general use of Lintner's improved method may therefore be expected and, in the writer's opinion, the results hitherto published by Power, Tutin and Rogerson are insufficient to provoke a contrary result. These authors have stated[1] that "such methods for the valuation of hops as are based on the titration of extracts obtained by means of light petroleum and similar solvents are of very doubtful utility" because "the resinous material contains a large proportion of fatty acids and their esters."   This criticism,

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[1] *Trans.*, 1913, 103, 1292.

if sound, would apply to the gravimetric method of Briant and Meacham no less than to the volumetric method of Lintner, and, since Power and his collaborators offer us nothing in place of these methods, would leave us without any chemical method of estimating the preservative value of hops. On pages 550 to 552 the work of Power and his collaborators, which covers many substances beyond the bitters and resins of hops, is briefly referred to, and grounds are there stated for regarding as far from complete their alleged proof that an ordinary petroleum ether extract of hops contains notable amounts of fatty acids. That the "soft resins" of hops, as ordinarily obtained, were a mixture of at least two resins and two crystalline acids, together with traces of essential oil and wax, was generally recognised, but, as Chapman pointed out[1] in discussing the paper of Power and his collaborators, the analytical methods which they regarded as of very doubtful utility had in fact done very good service from the technical point of view. Among German critics of Power, Tutin and Rogerson may be cited O. Neumann,[2] who states that their conclusions are contrary to established experience, which leaves no doubt as to the utility of the methods they condemn. The writer need do no more than state that, so far as he can learn, these methods remain in general use.

That in future less reliance must be placed on the single figure for total soft resins as a measure of the value of hops, and that much more general use ought to be made of Lintner's method of discriminating between the α- and β-resins (Vol. VII, pp. 177–178) follows from the work of Brown and Clubb, referred to on pages 552 to 554. If sufficient workers could be found to make such an extended chemical analysis and at the same time to make use of the biological method of Brown and Ward for the direct estimation of preservative power, it might well be that some simple expression would be found to correlate the chemical and biological results. In such an event, the biological method might be expected to supersede chemical methods in brewing laboratories, since it requires less time, whilst chemical methods would no doubt be preferred by those only occasionally concerned with hop analysis, owing to the inconvenience of maintaining cultures of *Bacterium X* and culture media over long periods.

Direct Estimation of the Antiseptic Power of Hops.—Comparatively little progress has been made towards a general adoption of the method of Brown and Ward, outlined on pages 179–181 of Vol. VII. The method, however, assumes greatly increased importance since the publication of the work of Brown and Clubb (pages 552 to 554). Still greater importance may attach to it if Power and his collaborators should, as a result of further research, justify the aspersions they have cast on all the chemical methods of valuing hops.

It was pointed out in Vol. VII that, before the method of Brown and

[1] *Proc. Chem. Soc.*, 1913, 29, 181.
[2] *Chem. Zeit.*, 1913, 37, 1317.

Ward could pass into general use, it would need to be approximated to the Rideal-Walker method of testing disinfectants by using as standard a solution of some antiseptic of definite composition. Brown and Clubb have since published data[1] which show that salicylic acid would serve for this purpose and, in discussing their paper, the writer[2] made some definite suggestions for expressing all results in terms of salicylic acid. The *Journal of the Institute of Brewing* should be watched for further developments of the method of Brown and his collaborators, but meanwhile reference to the paper of Brown and Clubb (*loc. cit.*) and to the discussion thereon will enable anyone to use the method and to express his results in such a way that they will be comparable with those of other workers and convertible into terms of any standard that may ultimately be agreed upon. The writer's contribution to the discussion was made without a full knowledge of Brown and Clubb's results and under the supposition that the β-resin was more toxic than the α-resin. As described in the preceding paragraphs, Brown and Clubb have shown that the α-resin is the more toxic, but this in no way affects the writer's suggestions for standardising the method of Brown and Ward.

Estimation of the Bitterness of Hops.—Since the above was written, a paper[3] has appeared entitled the Quantitative Determination of the Resins in Hops. Although possessing the authority which must attach to any paper issuing from the Carlsberg Laboratory, the writer prefers to introduce it under the above heading, as the method does not measure the total content of resins, nor that of soft resins. Evidence is, however, adduced that it affords an approximate measure of the bitterness of hops. It is an expeditious method, consisting essentially in extracting the total resins with cold ether and titrating the extract with alcoholic potash. It assumes that the hard resin is bitter—a fact the authors claim to have proved—but less bitter than the soft resins, a fact which is compensated in this method by its much greater equivalent. The method is as follows:

The hops are put through a mincing machine and 5 grm. are transferred to a 300 c.c. flask and dried in a vacuum for 24 hours at 35° C. The dried material is then covered with 150 c.c. of absolute ether and left for 1 hour with repeated shaking. The liquid is then filtered and the residue washed with 100 c.c. of ether. Finally the filtrate is titrated with $N/20$ alcoholic (93%) potassium hydroxide, using 6 to 8 drops of 1% phenolphthaleïn as indicator, the titration being continued until further additions of alkali no longer increase the colour intensity. Each c.c. of $N/20$ potassium hydroxide corresponds to 0.02 grm. resin. This factor is only exact for the β-resin; the γ-resin has a much higher equivalent, but, as the bitterness of the resins is approximately proportional to the reciprocals of their equivalents, the use of this factor approximately measures the bitterness of the hops. The previous drying and the use of

[1] *J. Inst. Brewing,* 1913, 19, 272–274.
[2] *Ibid.,* 290.
[3] O. Winge and J. P. H. Jensen, *Compt. rend. du Lab. de Carlsberg,* 1914, 11, 116.

absolute ether are essential, as otherwise tannins pass into solution and introduce errors. Even with hops containing so little as 4.5% of water and with absolute ether, the resins may be overestimated by 1% (on the hops).

The above-quoted paper criticises adversely all previous methods for the valuation of hops, especially such as depend on differentiation of the hard and soft resins. The authors' discovery that the hard resin does confer a bitter flavour on liquids boiled with it is the basis of this criticism, but they seem to overlook the fact that British workers at least have been more concerned to measure the preservative properties than the bittering properties of hops. So far as the writer is aware, there is no evidence that the hard resin has any preservative value. Nor does he know of any evidence for the authors' statement that the resin produced by the oxidation and polymerisation of hop oil is identical with the γ-resin. Hayduck, it is true, expressed this view in 1888, but he adduced no evidence, and such as has accumulated since leads to a contrary conclusion.

Estimation of Arsenic in Hops.—In Vol. VII, p. 186, directions were given for the destruction of organic matter as a preliminary to the estimation of arsenic, and it was stated that if this were omitted, arsenic might be underestimated. In reviewing Vol. VII, A. C. Chapman[1] stated that, in his experience, higher results were, as a rule, obtained when working directly on the hops than when the organic matter was destroyed. The writer takes this opportunity of stating that his personal experience accords with that of Chapman. Having tried every method for the destructon of organic matter which he has seen recommended, he has never recovered more arsenic after such treatment than when it was omitted, and sometimes he has found less, but in writing for Vol. VII he did not feel justified in giving his own view which he knew conflicted with the experience of many chemists constantly occupied with the examination of brewing materials.

[1] *Analyst*, 1913, 38, 599.

ANIMAL BASES.

BY K. GEORGE FALK, PH.D.

Ninhydrin Test.—The conditions for carrying out the triketohydrindene hydrate, $C_6H_4\diagdown_{CO}^{CO}\diagup C(OH)_2$, (*ninhydrin*[1]) test for the presence of amino-acids, peptides, peptones, proteins, etc., have been studied in detail.[2] In carrying out this test, 2–10 c.c. of the solution in question are boiled for 1 minute with 0.2 c.c. of a 1% aqueous triketohydrindene hydrate solution The appearance of a blue colour shows the presence of a substance containing a carboxyl and an amino-group attached to an aliphatic radical. Certain precautions are necessary. The solutions must be neutral. A blue colouration will appear if a strongly alkaline ninhydrin solution is warmed but this colour disappears on dilution. Since the coloured substance obtained is colloidal and sensitive towards electrolytes (cations exerting the predominating action), only small amounts of neutral salts may be present. The test is given most readily if the amino-group is in the α position to the carboxyl group; if it is in the β, γ, δ, ϵ, positions, the blue colour appears only on heating. Fairly concentrated solutions of alcohols, aldehydes, ketones, and reducing sugars give a red or blue colouration when warmed with a ninhydrin solution; the colour is intensified by the addition of alkali and is said to be different from that obtained with amino-acids, etc. Addition of alkali to the test with the latter after the colour has developed, does not dissipate the colour. The reagent is sensitive for the following substances in the dilutions indicated: Glycocoll, 1:65,000; *d*-alanine, 1:26,000; *d*-valine, 1:15,000; *l*-leucine, 1:25,000; *d*-glutamic acid, 1:22,000; asparagine, 1:19,000; *dl*-phenylalanine, 1:26,000 *l*-histidine, 1:79,000; α-aminobutyric acid 1:16,000.

van Slyke Apparatus.—The apparatus devised by van Slyke for quantitatively estimating amino-groups by the reaction with nitrous acid and measurement of the nitrogen evolved has been improved in a number of particulars. For shaking the deaminising bulb and the Hempel pipette a motor is used. The apparatus is constructed with rubber connections and outflow tubes so that now it can be used an indefinite number of times without disconnecting any of its parts.[3] The mechanical shaking device increases the convenience, speed, and reliability of the results. To prevent foaming of viscous solutions

[1] Put on the market by Meister Lucius and Brüning, Höchst a.M.
[2] Paul E. Howe, *Biochem. Bulletin*, 1914, **3**, 269; Abderhalden and Schmidt, *Z. physiol. chem.* **85**, 143; E. Herzfeld, *Biochem. Zeit.*, **59**, 249; Halle, Loewenstein and Pribram, *Ibid.*, **55**, 357.
[3] It may be obtained with or without motor from Emil Greiner, 45 Cliff St., New York, or from Robert Goetze, 4 Hörtelstrasse, Leipzig. In England it is made by Müller, Orme & Co.

during the shaking, the addition of caprylic alcohol (Kahlbaum's "octyl alkohol (secundär)I") is recommended in place of the amyl alcohol originally suggested. A useful table for the conversion of c.c. of nitrogen gas into milligrams of amino-nitrogen at various temperatures and pressures is appended to this paper.[1] In a later paper[2] van Slyke describes a micro-form of this apparatus which answers almost all the requirements of ordinary work. The gas burette in this form holds 10 c.c., the upper part measuring the first 2 c.c. is 4 mm. in diameter and is divided into divisions of 0.02 c.c., the remainder is wider and divided into 0.05 c.c. The deaminising bulb has a volume of 11 to 12 c.c., and the burette of 2 c.c. 10 c.c. of nitrite solution and 2.5 c.c. acetic acid are required for each analysis and the correction for the reagents is 0.06–0.12 c.c. Only 0.5 mg. of amino-nitrogen is required for an analysis accurate to within 1%. The manipulations are the same as with the larger apparatus except that in the first stage of the analysis in freeing the apparatus from air the deaminising bulb should be shaken by the motor at a very high speed about as fast as the eye can follow. In the third stage when the nitric oxide is absorbed by the permanganate, the Hempel pipette should be shaken not faster than twice per second.

Sörensen's Formaldehyde Method.—The estimation of amino-acid nitrogen in fluids such as urine by the Sörensen formaldehyde method (titration with alkali after addition of neutralised formaldehyde solution) has been modified by using phosphotungstic acid to remove ammonia and other impurities.[3] The method is as follows: 200 c.c. of urine (24 hours' specimen diluted to 2,000 c.c.) are measured into a 500 c.c. Erlenmeyer flask, an equal quantity of 10% phosphotungstic acid (Merck's) in 2% hydrochloric acid added, allowed to stand at least 3 hours, 250 c.c. clear liquid decanted, 1 c.c. of a 0.5% phenolphthaleïn solution added, and then barium hydroxide a little at a time until the whole fluid turns decidedly pink. The mixture is allowed to stand 1 hour, two 100 c.c. samples (= 50 c.c. urine) are filtered off, neutralised to litmus with $N/5$ hydrochloric acid, 10–20 c.c. of neutralised formaldehyde solution added, and titrated to a deep red colour.[4] The result is corrected by deducting the amount of $N/10$ sodium hydroxide necessary to produce the same depth of colour in an equal quantity of water, freed from carbon dioxide, to which the same quantity of neutral formaldehyde has been added.

Esterification Method.—The separation of amino-acids by Fischer's ester method is not quantitative, as is well known. Loss occurs at each operation —esterification, distillation, and saponification. Some evidence has been obtained as to the percentage loss under the best conditions of alkali, etc., by experienced workers, starting with pure amino-acids. The percentage yields obtained after carrying through the three operations were as follows:

[1] J. Biol. Chem., 1912, 12, 275.
[2] J. Biol. Chem., 1913, 16, 122.
[3] S. R. Benedict and J. R. Murlin, J. Biol. Chem., 1913, 16, 385.
[4] Abderhalden and Weil, Z. physiol. Chem., 77, 59; Osborne and Jones, Am. J. Physiol., 26, 212.

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glycocoll 65%; d-alanine 64%; l-leucine 75% (in another case 88.8%); d-valine 68%; l-phenylalanine 54%; l-proline 69.5%; aspartic acid, about 60%; glutamic acid, about 70% (in another case 85%). In the hydrolysed protein material, which is separated by fractional distillation of the esters, it has been suggested that the fraction which formerly was distilled above 110° at less than 1 mm. pressure be worked up directly without distillation, this fraction being dealt with as easily in this way as by the old method.[1] The results of amino-acid distillations compared with the figures obtained by the hydrolysis of proteins indicate that most, if not all, of the fragments of the more common proteins have already been isolated, the losses being accounted for in the different manipulations.

Amino-acid Picrolonates.—The picrolonates of a number of amino-acids have been prepared.[2]   They may be obtained by dissolving molecular proportions of amino-acid and picrolonic acid in a small amount of warm water and allowing to crystallise.   Many of them are insoluble in cold water but much more readily soluble in alcohol.   The following list gives the solubility of the picrolonates of the corresponding amino-acids in grm. per 100 c.c. of water at 20°–23°, and also the melting points: dl-phenylalanine, 0.12, 212° (decomp.); tyrosine, 0.29, blackens at 260°; l-phenylalanine, 0.34, 208°; dl-leucine, 0.53, indefinite above 140°; l-leucine, 0.55, indefinite at about 150°; d-isoleucine, 0.58, 170°; dl-valine, 0.81, indefinite above 150°; dl-serine, 0.98, decomposes 265°; glycine, 0.99, 214°; dl-alanine, 1.01, 216°; d-valine, 1.20, 180°; d-alanine, 1.61, 214°; dl-aspartic acid, 1.69, blackens at 130°; dl-glutamic acid, 2.37, 194°.   Proline and oxyproline do not yield picrolonates readily under these conditions.   Separations may be effected with these salts; for instance, phenylalanine can be separated from glutamic and aspartic acids by adding enough picrolonic acid to combine with the former, when its picrolonate will crystallise out pure.

Separation of d-Valine and d-Alanine.—The ester method of separating amino-acids obtained from protein hydrolysis gives, by distillation and crystallisation, a number of fractions and subfractions containing two or more amino-acids which are difficult to separate in the ordinary way.   A method for separating d-alanine (and glycine, if present) and d-valine, which are obtained in one of these fractions, has been devised by Levene and van Slyke.[3] The method is based on the fact that a crystalline salt is formed by d-alanine with phosphotungstic acid in a ratio of 1 to 14 by weight and solubility 0.15 grm. alanine per 100 c.c. in a solution containing 20 grm. phosphotungstic acid in 100 c.c. 10% sulphuric acid, while under the same conditions the solubility of d-valine is 1.21 grm. per 100 c.c.   By alternate crystallisation of valine as the free amino-acid (with the addition of acetone to a concentration of 50–60%), and of alanine as the phosphotungstate, subsequently remov-

[1] Osborne and Jones, loc. cit.
[2] Levene and van Slyke, Proc. Soc. Exp. Biol. Med., 9, 111
[3] J. Biol. Chem., 1913, 16, 103.

ing the phosphotungstic acid with lead acetate, a practically quantitative separation of a mixture of the two amino-acids can be effected.

Separation of Cystine and Tyrosine.—A method has been devised[1] for separating cystine and tyrosine from each other (see Vol. VIII, Appendix, p. 689). The mixture is warmed with absolute alcohol saturated with hydrochloric acid and an equal volume of alcohol then added. Cystine is insoluble and may be filtered off, washed, dried, and weighed. Tyrosine readily forms the ester under these conditions and goes into solution. It may be recovered by diluting with 2 volumes of water, boiling for 8 hours and neutralising with ammonia.

Tyrosine.—Tyrosine in the free condition or in peptide combination may be estimated colourimetrically as follows:[2]

1 grm. of the dry protein is accurately weighed out and transferred to a 500 c.c. Kjeldahl flask, 25 c.c. of 20% hydrochloric acid are then added, the flask closed by means of a Hopkins condenser made from a large test-tube, and the contents of the flask boiled for 12 hours over a microburner. At the end of this time the flame is removed, the contents of the flask transferred on cooling to a 100 c.c. volumetric flask and made up to volume. 1 or 2 c.c. of this solution are then transferred to a 100 c.c. volumetric flask, 5 c.c. of the tyrosine reagent[3] added, and after 5 minutes 25 c.c. of a saturated solution of sodium carbonate, and the mixture then made up to 100 c.c. with cold tap water. The maximum colour (blue) develops in about 10 minutes. Therefore the reading should not be made before this time has elapsed. Fading is very slow in the presence of the large excess of reagent used. As nearly at the same time as possible a standard is prepared by treating 1 mg. of pure tyrosine with 5 c.c. of the phosphotungstic-phosphomolybdic reagent, then adding 25 c.c. of saturated sodium carbonate solution and making up to volume. The colours are compared by means of a Duboscq colourimeter, the standard solution being placed at 20 mm. As a standard solution, a solution of pure tyrosine in $N/10$ hydrochloric acid is used of such a concentration that 5 c.c. contain 1 mg. of tyrosine. In making the comparison of colour both solutions should of course be absolutely clear and contain no trace of precipitate; if any cloudiness is observed the solution should be filtered before being used.

Tryptophane and hydroxytryptophane also have been observed to give a slowly developing blue colour with this reagent.[4]

Tryptophane.—A quantitative method has been proposed for tryptophane based upon the blue colour developed slowly (30 hours) with a solution containing 20 grm. of p-dimethylaminobenzaldehyde, 500 c.c. concentrated hydrochloric acid, and 500 c.c. water. For colour comparison, an ammoniacal copper sulphate solution is used.[5] It is said that 0.1 mg. of tryptophane may be determined in 100 c.c. solution in this way.

Betaine.—A new process of obtaining betaïne hydrochloride from molasses residues was described by Stoltzenberg.[6] It depends upon the changes

[1] Plimmer, *Biochem. J.*, 1913, 7, 311.
[2] Folin and Denis, *J. Biol. Chem.*, 12, 245; 14, 457.
[3] A solution containing 10 % of sodium tungstate, 2 % of phosphomolybdic acid and 10 % of phosphoric acid is made up by adding 100 grm. of sodium tungstate, 20 grm. of phosphomolybdic acid, and 50 c.c. of 85% phosphoric acid to 750 grm. water, boiling for 2 hours with a reflux condenser, cooling, and diluting to 1000 c.c.
[4] Abderhalden, *J. Biol. Chem.*, 15, 357; *Z. physiol. Chem.*, 83, 468; 85, 91.
[5] Herzfeld, *Biochem. Zeit.*, 1913, 56, 258.
[6] *Ber.*, 1912, 45, 2248.

in solubility produced by saturating solutions with hydrochloric acid; the solubility of potassium chloride decreases from 34 to 1.9 parts per 100 at 20°, that of glutamic acid hydrochloride from 38 to 1.4, whilst that of betaïne hydrochloride increases from 40 to 41 parts. In a mixture of the three, the latter has a salting out effect on the other two.

Urea.—The quantitative estimation of urea may be carried out very readily by means of soja beans. The latter contain a urease which converts quantitatively the urea into carbon dioxide and ammonia (compare footnote, Vol. VII, p. 300). The estimation of the ammonia may then be carried out in the usual way, either by distillation into standard acid or by direct titration with a suitable indicator (methyl-orange). The method employed by Plimmer and Skelton[1] for the quantitative estimation of urea, and indirectly of allantoin, in urine is the simplest and easiest to carry out. It may best be described in their own words: "In its simplest features the method is no more than Folin's method[2] of estimating ammonia in urine. By fitting together three or four cylinders and Allihn bottles in series with a sulphuric acid bottle at the end, duplicate estimations of ammonia and urea in urine can be carried out simultaneously. In the cylinders for the urea estimations are put 50 to 60 c.c. of water, 1 grm. of finely ground soja beans and 5 (or 10) c.c. of urine. These cylinders are kept in a water-bath at a temperature of 35°–40° and an air current is drawn through the series. After about an hour the rubber connections between the cylinders and bottles are disjointed and 1 grm. of anhydrous sodium carbonate is dropped into the cylinders; they are then connected together again and the air current drawn through for another hour. To prevent frothing, liquid paraffin B. P. has been used; it is superior to petroleum and toluene as it does not evaporate and it obviates the necessity of using a tube containing cotton wool between the cylinder and Allihn bottle. It is not necessary to carry out a blank experiment with soja bean alone, since no ammonia was evolved by two different samples of the bean which were tested several times. The Allihn bottles are charged with excess of $N/10$ sulphuric acid (25 or 50 c.c.) which is titrated with $N/10$ alkali, using Alizarin Red as indicator. . . .

"Not only urea, but also allantoin, is decomposed by the magnesium chloride method of Folin. . . . Since urease has no action upon allantoin, the two substances can therefore be readily estimated in urines which contain both compounds; the difference between the two data will give the amount of allantoin."

The estimation of urea in other fluids may be carried out similarly. Several different methods have been described for the estimation of urea by means of the soja bean urease (E. K. Marshall, Jr., van Slyke, etc.), all based upon the same principle and differing only in some of the manipula-

[1] Biochem. J., 1914, 8, 70.
[2] Z. physiol. Chem., 37, 161.

tions. Solid preparations from soja beans containing the active enzyme are on the market under the names of Arlco-urease and Urease-Dunning. The specific character of the soja bean urease also makes it a convenient reagent for getting rid of urea as such in solutions which are to be used for other purposes. At the present time it appears as though this method of estimating urea is replacing the former methods.

Creatine; Creatinine.—The preparation of creatine and creatinine from urine in considerable quantities can now be carried out by the process developed by Benedict.[1] The method is as follows: To each 1,000 c.c. of urine (which must not be decomposed) 18 grm. of picric acid are added. It does not pay to work with less than 10 litres of urine. The picric acid is dissolved in boiling alcohol (40 grm. to 100 c.c.) and the hot solution is added with stirring. The mixture is allowed to stand over night and the supernatant fluid is siphoned off. The residue is poured upon a large Buchner funnel, drained by suction and washed once or twice with cold saturated picric acid solution and sucked dry. The dry, or nearly dry, picrate is treated in a large mortar or evaporating dish with enough concentrated hydrochloric acid to form a moderately thin paste (about 60 c.c. of acid for each 100 grm. picrate), and the mixture thoroughly stirred with a pestle for 3 to 5 minutes. The mixture is then filtered by suction on a hardened paper, the residue washed twice with enough water to cover it, and sucked as nearly dry as possible each time. The filtrate is at once transferred to a large flask and neutralised with an excess of solid magnesium oxide added in small portions, cooling the flask under running water. When all the hydrochloric acid has been neutralised the mixture will turn bright lemon yellow, or litmus paper may be used to test it. The mixture is then filtered by suction and the residue washed twice with water. The filtrate is at once strongly acidified with a few c.c. of glacial acetic acid, and (paying no attention to a precipitate which may form at this point) the solution is diluted with about 4 volumes of 95% alcohol and filtered by suction any time more than 15 minutes after a slight precipitate (chiefly calcium sulphate) has formed. The final filtrate is treated with 30% zinc chloride solution, using 3–4 c.c. for each 1000 c.c. of urine originally used. This mixture is stirred (a precipitate should form almost immediately) and allowed to stand overnight in a cool place. The supernatant fluid is then poured off and the precipitated creatinine zinc chloride collected on a Buchner funnel, washed once with water, then thoroughly with 50% alcohol, and finally with 95% alcohol and dried. The product should be a nearly white, light crystalline powder. 90 to 95% of the creatinine originally present should be recovered. Ordinarily 1.5 to 1.8 grm. of the double salt should be obtained per 1000 c.c. of urine used. To prepare creatine from the double salt, 100 grm. of the latter are treated with 700 c.c. of water in a large casserole and heated to boiling. 150 grm. of pure calcium hydroxide are then added

[1] *J. Biol. Chem.*, 1914, **18**, 183.

with stirring and the mixture boiled gently for 20 minutes (with occasional stirring). The hot mixture is filtered by suction and the residue washed with hot water. The filtrate is treated with hydrogen sulphide gas for a few minutes and poured through a folded filter to remove the zinc. The filtrate is acidified with about 5 c.c. of glacial acetic acid and boiled down rapidly to a volume of about 200 c.c. This solution is allowed to stand over-night in a cool place. The crystallised creatine is filtered off with suction, washed with a very little cold water, and then thoroughly with alcohol and dried. (The filtrate obtained at this point should be diluted with alcohol and treated with zinc chloride (50 c.c. of a 30% solution) for the recovery of unconverted creatinine.) This product is recrystallised by dissolving in about seven times its weight of boiling water, allowing the solution to cool slowly and then to stand for some hours. The crystallised product should be filtered off, washed with alcohol and ether and dried in the air for about half an hour. Thus obtained, the creatine contains water of crystallisation which it loses very readily upon exposure to air. To prepare creatine which can be weighed with exactness, it is necessary to dehydrate this product by heating for some hours at about 95°. The yield in this process is about 18 grm. of recrystallised creatine and about 55 grm. recovered creatinine zinc chloride. To prepare pure creatinine from the double salt, the latter, finely powdered, is placed in a dry flask and treated with seven times its weight (by volume) of concentrated aqueous ammonia. The mixture is warmed slightly and gently agitated until a clear solution is obtained, care being taken to drive off no more ammonia during the warming than is necessary to obtain a clear solution. The flask is stoppered, cooled, and placed in an ice-box for an hour or more. Pure creatinine crystallises out. The yield is 60–80% of the theoretical. If the product is coloured slightly yellow, it may be recrystallised either from boiling alcohol or by dissolving in five times its weight (by volume) of concentrated ammonia (warming enough to effect solution) and letting the solution stand in a cold place for some hours. Recrystallisation is usually unnecessary.

For the quantitative estimation of creatinine by Folin's method (yellow colour with picric acid and alkali), it is now recommended to replace the bichromate solution used as standard by a standard creatinine solution.[1] The creatinine-zinc chloride double salt may be used and is obtained pure by three recrystallisations from 10 parts of boiling 25% acetic acid and addition of 1 part concentrated alcoholic zinc chloride solution and 1.5 parts alcohol. 1.6106 grm. of the double salt dissolved in 1000 c.c. of $N/10$ hydrochloric acid gives a solution containing 1 mg. creatinine per c.c. Folin has given detailed directions[2] to carry out the estimation of creatine and creatinine in urine, blood, milk, tissues, muscles, etc. For blood or milk the method is as follows: 10 c.c. of the fluid are placed in a 50 c.c. glass-stoppered shaking

[1] Folin, J. Biol. Chem., 1914, 17, 463.
[2] J. Biol. Chem., 1914, 17, 469, 473.

cylinder, filled to the mark with saturated picric acid solution and shaken a few times. About 1 grm. of dry picric acid is added and the mixture shaken for 5 minutes, transferred to centrifuge tubes, the sediment and precipitate shaken down and the supernatant fluid poured through a filter. (If enough substance is available, the quantities taken may be doubled and filtered without preliminary centrifuging.) Proteins are removed by this treatment and the creatine and creatinine obtained in the filtrate. The standard solution to be used contains 0.2 mg. creatinine per 100 c.c.; it is prepared by diluting the standard solution described above with saturated picric acid solution. 5 c.c. of 10% sodium hydroxide solution are added to both the unknown solution and 100 c.c. of the standard solution; the solutions are allowed to stand 10 minutes and compared in a Duboscq colourimeter. Neither solution may contain more than $1\frac{1}{2}$ times as much creatinine as the other, otherwise the comparisons of colour are not reliable. In collecting blood for this determination, 10 drops of a 20% potassium oxalate solution are sufficient to prevent clotting in 30 c.c. of blood. To estimate both creatine and creatinine in blood or milk, 10 c.c. of the filtrate from 10 c.c. of blood and picric acid solution are heated in an autoclave at 120° for 20 minutes in a flask covered with tin-foil, cooled, diluted to 25 c.c. with saturated picric acid solution, 1.25 c.c. of 10% sodium hydroxide solution added and the colour compared with standard solutions containing 0.5, 1 and 2 mg. of creatinine in 100 c.c. saturated picric acid solution, to 20 c.c. of each of which 1 c.c. of 10% sodium hydroxide solution had been added. With the standard set at 10 mm.,

$$\frac{10 \times 125 \times 0.5 \text{ (or 1 or 2)}}{\text{reading of unknown}} = \text{mg. creatine plus creatinine in 100 c.c. blood}$$

or milk.

For the estimation of creatine and creatinine in the other substances, similar methods are employed with slight modifications in the procedure depending upon the properties of the material. For creatine plus creatinine in urine, a convenient method is the following (Benedict[1]): Such a volume of urine as will contain between 7 and 12 mg. of total creatinine is introduced into a small flask or beaker and from 10 to 20 c.c. of N-hydrochloric acid added together with a pinch or two of powdered or granulated lead. The mixture is boiled over a free flame as slowly or as rapidly as may be desired until very nearly down to dryness, when the heating should be continued to dryness either on the water-bath or holding the vessel and heating carefully for a moment or two. The residue should best stand on the water-bath for a few minutes until most of the excess of hydrogen chloride has been expelled, after which it is dissolved in about 10 c.c. of hot water and the solution rinsed quantitatively through a plug of cotton or glass-wool (to remove all metallic lead) into a 500 c.c. volumetric flask. 20-25 c.c. of saturated picric acid solution are added and about 7-8 c.c. of 10%

[1] *J. Biol. Chem.*, 1914, **18**, 192.

sodium hydroxide solution which contains 5% Rochelle salt (to prevent any formation of turbidity, due to dissolved lead—it has no effect upon the creatinine readings). The flask is filled to the mark at the end of 5 minutes and the colour comparisons made as usual. For creatinine in urine (Folin) 1 c.c. of the standard solution is measured into a 100 c.c. volumetric flask, 1 c.c. of urine into another, 20 c.c. of saturated picric acid added to each and then 1.5 c.c. of 10% sodium hydroxide solution. After 10 minutes the flasks are filled to the mark and the colour of the solutions compared. The special 1 c.c. pipettes, accurate to 0.1%, may be obtained from Eimer and Amend, New York. In estimating creatinine in urine, the colour due to sugar and picric acid appears too slowly to interfere, especially in the cold. β-hydroxybutyric acid, at least in the amounts ordinarily present, does not interfere. Acetone and acetoacetic acid interfere markedly and must be removed; acetone by aeration, acetoacetic acid by extraction with ether and subsequent aeration, or by distillation *in vacuo* at temperatures below 65° in the presence of some phosphoric acid.[1] For creatine plus creatinine in diabetic urines, the autoclave method or evaporation to dry- ness cannot be used; Folin's original method of heating on the water-bath during 3–4 hours with hydrochloric acid is the only safe process; a little evaporation should be permitted to remove the acetone.

To estimate creatine and creatinine in muscle or other tissues, a prelimi- nary treatment with picric acid solution removes the protein substances as insoluble picrates.

Adenine.—A method of obtaining adenine from molasses residues has been described by K. Andrlik.[2] They are treated with copper sulphate and sodium hydroxide, the precipitate obtained being decomposed in sus- pension with hydrogen sulphide and filtered. The filtrate upon evaporation gives crystals of adenine which may be purified by recrystallisation (animal charcoal). 20 grm. of adenine were obtained from 40 kilos of molasses residues.

[1] Greenwald, *J. Biol. Chem.*, 1913, 14, 87.
[2] *Zeit Zuckerind, Böhmen*, 34, 567.

ANIMAL ACIDS.

By PHILIP B. HAWK, M.S., Ph.D.

Kynurenic Acid.

In addition to occurring in the urine of the dog this aromatic hydroxy-acid has been found by Swain[1] in the urine of the coyote. To isolate the acid from urine proceed as follows: Acidify with hydrochloric acid in the proportion 1:25. From the acid fluid both the uric acid and the kynurenic acid separate in the course of 24–48 hours. Filter off the crystalline deposit of the two acids, dissolve the kynurenic acid in dilute ammonia (uric acid is insoluble) and reprecipitate it with hydrochloric acid. Capaldi[2] has proposed a method of estimating kynurenic acid quantitatively.

Homogentisic Acid.

Alkaptonurics do not possess the power to rupture the benzene nucleus of tyrosine and phenylalanine but eliminate this nucleus in the urine in the form of homogentisic acid. Normal persons disintegrate the nucleus and excrete it as carbon dioxide and water. Abderhalden was able to produce experimental *alkaptonuria* by feeding a normal man with 50 grm. of tyrosine. Garrod classes alkaptonuria, cystinuria, albinism and pentosuria as *inborn errors of metabolism*. The properties and reactions of homogentisic acid are discussed by Mörner.[3]

Hippuric Acid.

Lewis[4] has demonstrated that the ingestion of 6–10 grm. of sodium benzoate by a normal man is followed by the excretion in the urine of 85–90% of this benzoate in the form of hippuric acid.

Two very satisfactory methods (one volumetric and one gravimetric) for the quantitative estimation of hippuric acid have been proposed by Dakin (see "*Practical Physiological Chemistry*" (Hawk), 5th Ed. p. 520). One of the best of the more recent methods is that suggested by Folin and Flanders.[5]

The procedure with urine is as follows: To 100 c.c. of urine in a porce-

[1] *Am. J. Physiol.*, 1905, **13**, 30.
[2] *Zeit. physiol. Chem.*, **23**, 92.
[3] Upsala Läkare Föreningens Förhandlinger, **17**, 499.
[4] *J. Biol. Chem.*, 1914, **18**, 225.
[5] *J. Biol. Chem.*, 1912, **11**, 257.

lain evaporating dish add 10 c.c. of 5% sodium hydroxide and evaporate to dryness on a steam-bath (over night). Transfer the residue to a 500 c.c. Kjeldahl flask by means of 25 c.c. of water and 25 c.c. of concentrated nitric acid. Add 0.2 grm. of copper nitrate, a couple of pebbles or glass beads and boil very gently 4½ hours over a micro-burner. The flasks should be fitted with loosely fitting Hopkin's condensers, through which a good current of water should be flowing to prevent loss of benzoic acid or change in concentration of the nitric acid. After cooling, the condensers are rinsed down with 25 c.c. of water and the contents of the flask transferred to a 500 c.c. separating funnel by the use of 25 c.c. of water. The total volume of the solution should now be 100 c.c. Just enough ammonium sulphate is added to saturate the solution (about 55 grm.), which is extracted four times with washed chloroform using 50, 35, 25 and 25 c.c. portions. The successive portions of chloroform are collected in another separating funnel and treated with 100 c.c. of a saturated solution of sodium chloride to each litre of which has been added 0.5 c.c. of concentrated hydrochloric acid. The mixture is shaken well and the chloroform run into a dry 500 c.c. flask and titrated with $N/10$ sodium ethoxide in alcohol using 4–5 drops of phenolphthalein as indicator. The first distinct end-point should be taken. The sodium ethoxide is prepared by dissolving 2.3 grm. of cleaned sodium in 1,000 c.c. of absolute alcohol and may be standardised against pure benzoic acid in washed chloroform.

Haas[1] has devised the following test for the detection of hippuric acid: The material under examination (powder) in the presence of red phosphorus is covered with chloroform; bromine is then added in slight excess and the mixture warmed until a red solution results. A few c.c. of water are added, the bromine and chloroform expelled, the solution cooled, a little protein solution added and sulphuric acid poured down the side of the vessel. A purple zone then develops above the sulphuric acid layer.

Uric Acid.

The ingestion of 2-phenylquinoline-4-carboxylic acid (atophan) was shown by Nicolaier and Dohrn[2] to cause an increase in the output of uric acid. This finding has been verified by various workers (Weintraud; Frank and Bauch; Deutsch; Zuelzer; Retzlaff; Brugsch; Smith and Hawk and others). In normal cases and in non-gouty cases the uric-acid elimination, under the influence of atophan, is rather large the first day, and falls after a day or two to a point below normal. This is apparently due to the depletion of the supply of uric acid in the blood, and as no urates are deposited, there is no replenishment of the depleted blood. In the case of gout, however, there are deposits, which may be mobilised, tending to maintain the blood

[1] *Trans.*, 1912, 101, 1254.
[2] *Deut. Arch. f. inn. Med.*, 1908, 93, 331.

concentration, thus accounting for the rather high and long-continued increased excretion of uric acid noted in gout cases. Daniels[1] has shown an apparent increase in the uric acid output in gout, over that due to atophan, by the use of lithium. Abl[2] claims that calcium salts, barium sulphate and bismuth nitrate decrease uric acid excretion, whereas santonin, mustard, chloral hydrate, arsenic, strontium, etc., increase the output. This author also claims that the increase in uric-acid output secured by atophan ingestion is augmented by use of calcium salts, barium sulphate, uzara and atropine.

Interesting experiments on the specific rôle of foods in relation to the composition of the urine have recently been reported by Blatherwick.[3] Oranges, raisins, apples, bananas, cantaloups and potatoes, particularly the last two, are very effective in reducing the formation and excretion of acid. Tomatoes are less valuable. The cereals increase the formation of acids. Plums, prunes, and cranberries, in spite of their alkaline ash, increase the excretion of acid, owing to the benzoic acid which they contain. Meat produces a large increase in the acidity of the urine. An increase in the H^+ concentration is accompanied by an increase in titratable acidity and ammonia excretion. All acid urines are supersaturated with uric acid; all alkaline urines may dissolve more uric acid.

Estimation in Urine: Method of Folin and Denis.—From 1 to 2 c.c. of urine are measured into an ordinary centrifuge tube by means of a modified Ostwald pipette. A sufficient amount of distilled water is then added to bring the volume of liquid in the tube to about 5 c.c., 6 drops of 3% silver lactate solution, 2 drops of magnesia mixture, and a sufficient amount (10–20 drops) of concentrated ammonia to dissolve the silver chloride, are then added. The tube is now centrifuged for 1 or 2 minutes, the supernatant liquid poured off and to the residue in the bottom of the tube are added 1 drop[4] of concentrated hydrochloric acid and 5 or 6 drops of freshly prepared saturated hydrogen sulphide water, and the tube is placed in a beaker of boiling water until all excess of hydrogen sulphide has been driven off (usually about 5 minutes).

As hydrogen sulphide gives a blue colour with the "uric acid reagent," care must be taken to obtain its complete removal. To determine whether this has been accomplished, 1 drop of 0.5% lead acetate solution should be added to the contents of the tube after the latter has remained in the water-bath for about 5 minutes, and if any hydrogen sulphide remains a dark-brown precipitate will be formed. If this be the case the tube should be returned to the water-bath for further heating.

When all the excess of hydrogen sulphide has been driven off, the material is centrifuged for 1 or 2 minutes. The supernatant liquid is transferred by decantation to a small beaker, the residue washed in the tube (carefully so as

[1] *Arch. Int. Med.*, 1914, 13, 480.
[2] *Arch. exp. Path. Pharm.*, 1914, 74, 119.
[3] *Arch. Int. Med.*, 1914, 14, 409.
[4] Fine suggests that the single drop of hydrochloric acid is insufficient to make the solution acid and accordingly uses 2 or 3 drops.

to disturb the residue as little as possible) with two portions of 2 c.c. each of distilled water,[1] adding the washings to the material in the beaker.

To the solution containing the uric acid, 2 c.c. of the uric-acid reagent are added and 10 c.c. of saturated sodium carbonate solution, and the mixture is transferred to a 50 c.c. volumetric flask and made up to volume. The colour is then compared with that obtained from 5 c.c. of the standardised uric acid-formaldehyde solution which is treated with 2 c.c. of the uric-acid reagent and 10 c.c. of saturated sodium carbonate solution and diluted to 50 c.c.

In the case of urines containing much protein it will be found that after the addition of hydrogen sulphide the solution obtained is invariably of a brownish tint, which interferes with the colour comparison, and thus makes accurate readings very difficult. This difficulty can be overcome by adding to the hot solution (after removal of all hydrogen sulphide) from 2 to 10 drops of a 10% solution of sodium acetate. Unless albumin be present, sodium acetate should not be added as its presence tends to give slightly low results.

This procedure has also been found useful in estimating uric acid in blood where the same trouble is met with when, as occasionally happens, the protein has not been entirely removed.

Estimation in Blood: Method of Folin and Denis.—From 15 to 25 grm. of normal blood are needed for a determination. This is first treated to remove the protein, then concentrated, and the small volume, containing the uric acid, is treated as in the estimation in urine.

The blood is drawn into small, wide-mouthed bottles previously weighed and containing a small amount (about 0.1 grm.) of finely powdered potassium oxalate. From the subsequent weight of each bottle is obtained the weight of the blood. Five times this weight of $N/100$ acetic acid solution[2] is placed in an ordinary 1000 c.c. flask[4] and heated to boiling. The oxalated blood is then poured into this boiling acetic acid solution, stirring constantly, and the heating is continued until the solution has again begun to boil. The mixture is filtered while still hot. The coagulated material on the filter paper[3] is transferred back into the flask (by means of a small spoon or a spatula), about 200 c.c. of boiling water[4] are poured over it and it is allowed to stand for a few minutes. This mixture is then filtered through the same filter as was used for the first filtration. The filtrate in the receiving flask should be very nearly as clear as water, and will be found to be so if the original blood was promptly shaken with the oxalate so that no clotting has taken place.

If clotting has occurred, the coagulation and washing of the blood is a little more complicated. The clot leads to so much bumping in the boiling

[1] The washing of the precipitate of silver sulphide without disturbing it, is rather unsuccessful, so the tube may be centrifuged for a minute or two after each washing (Smith).

[2] Fine suggests that the pouring of blood into boiling $N/100$ acetic acid is quite apt to give coloured filtrates, and advises the use of about the same amount of boiling distilled water (i.e., 4 times the weight of blood), bringing this mixture to boiling and then adding enough $N/100$ acetic acid to bring about complete coagulation. About an equal amount is required.

[3] It was found that the transferring of coagulum could be avoided by using large (400 c.c.) casseroles, for the coagulation, and keeping the coagulum in the casserole rather than allowing it to go on to the filter (Smith).

[4] For this washing, water is used rather than $N/100$ acetic acid, because if the latter is used the coagulum will give off more or less of the blood pigment and the filtrates will be less clear.

acetic acid solution that it is not practical or safe to try to heat the mixture to boiling. The filtration is, therefore, made earlier. The partially coagulated clot is then broken up with a glass rod, transferred to a mortar, and there ground into a paste in the presence of hot water. This suspension is then poured upon the filter. The protein material on the filter is then washed, as before, with about 200 c.c. of hot water. In this case the combined filtrates are, however, never colourless but more or less reddish. On being heated to boiling a second small coagulum will be obtained and the filtrate will then be practically as clear as water.

The combined filtrate and washings, containing the uric acid and other soluble materials, are further acidified by the addition of 5 c.c. of 50% acetic acid, and are evaporated, over a free flame in a suitable dish,[1] to a very small volume (about 3 c.c.). The liquid is then poured into an ordinary centrifuge tube and the dish washed with two successive portions of 0.1% lithium carbonate solution, using about 2 c.c. for each rinsing. Any solid material adhering to the sides of the dish is removed by rubbing with a rubber tipped stirring rod and added to the solution in the centrifuge tube. This solid material can then be removed from the suspension in the tube by centrifuging—and pouring the supernatant liquid into another tube, washing the sediment with lithium carbonate solution (Smith).

The liquid in the centrifuge tube, which at this stage should not be more than 10 c.c. in volume, is then treated as in the method given for urine.

Preparation of the Uric Acid Standard.—The uric acid-formaldehyde solution is prepared as follows: 1 grm. of uric acid is placed in a 1,000 c.c. flask and dissolved by means of an excess of lithium carbonate (200 c.c. of a 0.4% solution). To the solution are added 40 c.c. of 40% formaldehyde solution, and the mixture is shaken and allowed to stand for a few minutes. The clear solution is acidified by adding 20 c.c. of N-acetic acid and the whole is diluted to the litre mark with water. The solution should remain perfectly clear and the next day (but not before) it can be standardised against a freshly prepared lithium carbonate solution of uric acid. The colour produced by 5 c.c. of the solution corresponds very nearly with the colour obtained from 1 mg. of uric acid. The colourimeter reading obtained for this solution, when thus compared against 1 mg. of pure uric acid is, of course, thereafter to be used as the standard value corresponding with 1 mg. of uric acid.

Preparation of the Uric Acid Reagent.—Add to 750 c.c. of distilled water in a 1,000 c.c. flask, 100 grm. of sodium tungstate, and 80 c.c. of phosphoric acid (85%); boil gently, under a reflux condenser, for about 2 hours. Dilute to a litre.

References.—Otto Folin and W. Denis, On the colourimetric determination of uric acid in urine, *J. Biol. Chem.*, 1913, **14**, 95.

[1] Deep (half globular) dishes 10 cm. in diameter and having a capacity of 250 c.c. are very good for this purpose. While free flames are the most convenient for concentrating the uric acid solutions care must, of course, be taken not to char the contents towards the end of the operation. Unless the solution can be watched carefully at this stage, it is safer to finish the concentration on the water-bath.

Otto Folin and W. Denis, Determination of uric acid in blood, *J. Biol. Chem.*, 1913, 13, 469.

Private communication from Morris S. Fine.

Smith and Hawk, unpublished data.

Benzoic Acid.

Benzoic acid is found in the urine of the rabbit and dog. In certain kidney diseases it may also occur in human urine.[1] The benzoic acid apparently arises from the fermentative decomposition of hippuric acid. The ingestion of benzoic acid or benzoates leads to an increased elimination of hippuric acid in the urine. This is due to the fact that within the body synthesis of hippuric acid occurs owing to a conjugation of glycocoll with the benzoic acid or benzoate ingested. This synthesis is believed to be brought about principallv by the kidney cells but may also occur elsewhere.[2]

Oxalic Acid.

Oxalic acid occurs in the urine in the form of calcium oxalate. When the oxalate content of the urine is increased from any cause the condition is called *oxaluria*. This calcium oxalate crystallizes in the form of dumb-bell or octahedral ("envelope") crystals. The origin of the calcium oxalate of the urine is not definitely known. It is eliminated at least in part, unchanged when ingested. Therefore, since many food substances (tomatoes, grapes, asparagus, lettuce, etc.) contain calcium oxalate, it seems likely that a portion of the urinary oxalate originates from the ingested food. Another portion of oxalic acid may be formed in the body in the course of the metabolism of fat and protein. Incomplete oxidation of carbohydrate material may also yield oxalic acid. To prepare calcium oxalate from the urine proceed according to one of the following methods:

First Method.—Place 200–250 c.c. of urine in a beaker, add 5 c.c. of a saturated solution of calcium chloride, make the urine slightly acid with acetic acid, and stand the beaker aside in a cool place for 24 hours. Examine the sediment under the microscope and compare the crystalline forms with those shown in Vol. VII, Fig. 26, p. 384.

Second Method.—Proceed as above, replacing the acetic acid by an excess of ammonium hydroxide and filtering off the precipitate of phosphates.

Chondroitin-sulphuric Acid.

This acid is found principally in cartilage from various sources. It may also be formed by the decomposition of chondromucoid, another cartilage constituent. Upon hydrolysing chondroitin-sulphuric acid by means of acid, a nitrogenous substance known as chondroitin is formed, and sulphuric

[1] Jaarsveld and Stokvis, *Arch. exp. Path. Pharm.*, 10.
[2] Kingsbury and Bell: *J. Biol. Chem.*, 1915, 21, 297.

acid results as a by-product. From chondroitin one may then obtain chondrosin, with acetic acid as a by-product. This chondrosin is a reducing substance. Schmiedeberg[1] has shown the formula for chondroitin-sulphuric acid to be $C_{18}H_{27}O_{17}NS$. The acid may be obtained in the form of an amorphous white powder which is very soluble in water. For methods of preparation see Schmiedeberg (loc. cit.) and Kondo.[2]

Colloidal Nitrogen.

The so-called "colloidal nitrogen" of the urine consists in large part of oxyproteic, alloxyproteic and autoxyproteic acids. This "colloidal nitrogen" may be precipitated by alcohol or basic lead acetate. It has been claimed that the urine of cancer patients contains abnormally large quantities of "colloidal nitrogen." This claim has not been absolutely substantiated. Some investigators[3] claim that the "colloidal nitrogen" precipitate contains uric acid, purine bases, etc., and is of no diagnostic significance.

Glycuronic Acid.

Biberfeld[4] claims that glycuronic acid cannot be an intermediary product in the metabolism of glucose, since in his experiments this acid was not utilised by the animal body, but when introduced intravenously was almost completely eliminated in the urine unchanged, or rather in conjugated form.

Amino-acids.

Recent experiments have made necessary a revision of our ideas regarding the nutritional relationship of these acids. According to the older views, the amino-acids which were formed as end-products of protein digestion in the intestine, were resynthesised in their passage through the walls of the intestine, and appeared in the circulating blood as blood proteins. Careful analysis of the blood failed to show the presence of any amino-acids. Recently, experiments by Folin and Denis, Buglia, Van Slyke and Meyer, and others, have yielded important evidence against the intestinal synthesis of amino-acids. We now know that amino-acids are present in the blood, and the only reason we were unable to detect them heretofore was because of the crudeness of the methods used. The method of Van Slyke, given in Vol. VII, p. 263, is particularly satisfactory for the estimation of amino-acids. The formaldehyde titration method of Sörensen and its modification by Henriques, are also of importance. The first workers who were successful in isolating amino-acids from the circulating blood were Abel and his associates, of Johns Hopkins University. They accomplished this by means of

[1] Arch. exp. Path. Pharm., 28.
[2] Biochem. Zeit., 26.
[3] de Bloeme, Swart and Terwen; Biochem. Zeit., 1914, 65, 345.
[4] Biochem. Zeit., 1914, 65, 479.

their so-called "artificial kidney," or vividiffusion apparatus. By means of this unique apparatus, the blood of a *living* animal may be subjected to dialysis for a period of several hours, and amino-acids, as well as other crystalloids, removed.

Acetone.

The quantitative estimation of acetone in the blood has recently assumed considerable importance, particularly in case of a pronounced acidosis, such as is frequently met with in diabetes mellitus. The blood of a normal person contains, at most, but a trace of acetone, although this amount may be greatly increased in certain pathological states. Marriott[1] found the blood of normal children to contain 0.06–0.08 mgm. of total acetone (acetone plus diacetic acid) per 100 c.c. In the case of a child in *coma*, the acetone value rose to 23.4. In the author's laboratory, Dr. Bergeim has demonstrated an acetone value of 48.65 mgm. per 100 c.c. of blood serum for the blood of an adult male diabetic in coma.

There are several methods in use for quantitatively estimating acetone in urine. These methods include those of Messinger, Messinger-Huppert, Scott-Wilson, and Folin. The method of the latter, as modified by Hart, for the estimation of total acetone (acetone plus diacetic acid) is as satisfactory as any. The method includes the transformation of the diacetic acid into acetone and carbon dioxide by means of heat; and the subsequent removal of the acetone thus formed, as well as the preformed acetone, by means of an air current as first suggested by Folin. The procedure is as follows: Introduce into a wide-mouthed bottle 200 c.c. of water, an accurately measured excess of $N/10$ iodine solution, and an excess of 40% potassium hydroxide. Prepare an aeration cylinder, containing alkaline hypoiodite solution, to absorb any acetone which may be present in the air of the laboratory, and suspend between this cylinder and the bottle (above referred to) a test-tube about 2 in. in diameter. This large test-tube should contain 20 c.c. of the urine under examination, 10 drops of a 10% solution of phosphoric acid, 10 grm. of sodium chloride, and a little petroleum, and should be raised sufficiently high to facilitate the easy application of heat to its bottom portion. The connections on the side of the tube should be provided with bulb-tubes containing cotton. When the apparatus is arranged as described, it should be connected with a Chapman pump and an air current passed through for 25 minutes. During this period the contents of the test-tube are heated just to the boiling-point, and after an interval of 5 minutes again heated in the same manner. By this means the diacetic acid is converted into acetone, and at the end of the 25-minute period this acetone, as well as the preformed acetone, will have been removed from the urine to the absorption bottle, and there retained as iodoform. The contents of the absorption bottle should now be acidified with concentrated

[1] *J. Biol. Chem.*, 1913, **16**, 293.

hydrochloric acid and titrated with $N/10$ sodium thiosulphate and starch. (For details and other methods, see *"Practical Physiological Chemistry"* (Hawk), 5th Ed., pp. 533-541).

The nephelometer may be used in estimating minute quantities of acetone as Marriott[1] has shown. Sobel[2] has suggested a quantitative process based on Lieben's test. The iodoform is changed into silver iodide by means of fuming nitric acid and silver nitrate.

Rosenbloom[3] claims that the presence of protein in urine renders inaccurate the detection of acetone by means of Lieben's test. The protein interferes with the production of iodoform, as well as with the deposition of iodoform crystals. It is necessary to distil the urine, and use the distillate in testing.

The test of Frommer[4] is a very satisfactory one. It is based on the fact that acetone reacts with salicylaldehyde to form dihydroxydibenzoylacetone. The chemistry of the test is explained in the following equations:

$$OH.C_6H_4.CHO + CH_3.CO.CH_3 \rightarrow OH.C_6H_4.CH:CH.CO.CH_3 + H_2O$$
$$\text{Salicylaldehyde} \qquad \text{Acetone}$$

$$OH.C_6H_4.CH:CH.CO.CH_3 + OH.C_6H_4.CHO$$
$$\rightarrow OH.C_6H_4.CH:CH.CO.CH:CH.C_6H_4.OH + H_2O$$
$$\text{Dihydroxydibenzoylacetone}$$
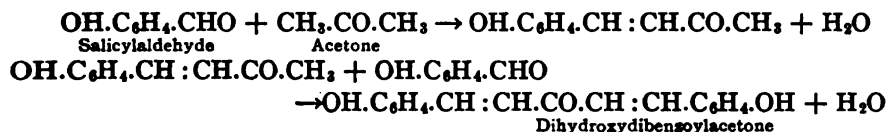
The chemistry of Legal's colour test, which depends upon the interaction of nitroprusside and acetone, has been studied by Cambi.[5] He claims that the colour reaction is due either to: (I) the formation of a complex ion of ferropentacyanide with the isonitroso compound of the ketone, or to (II) the formation of such an ion with the isonitroamine derivative of the ketone.

Aceto-acetic Acid.

The quantitative estimation of this acid, in urine and blood, is of considerable importance in connection with acidoses, such as may occur in diabetes mellitus and certain other disorders. The acid may be determined by the process embraced in the Folin-Hart method. The method is as follows: Arrange the apparatus as described under the Folin-Hart method for estimating acetone (see page 576). Start the air current in the usual way, and permit it to run 25 *minutes, without the application of heat to the urine under examination.* Under these conditions, the preformed acetone present in the solution is all removed. Immediately attach a freshly prepared absorption bottle containing alkaline hypoiodite solution. Apply heat to the large test-tube as already described *in order to convert the diacetic acid into acetone,* permit the air current to continue for the usual 25 minutes, and determine the diacetic acid value, in terms of acetone, by the usual

[1] *J. Biol. Chem.*, 1913, 16, 289.
[2] *Apoth. Ztg.*, 1914, 52, 62.
[3] *J. Am. Med. Assn.*, 59, 445.
[4] *Berl. Klin. Woch.*, 42, 1008.
[5] *Atti. accad. Lincei*, 1913, 22, I, 376.

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titration process. (For details and other methods, see "*Practical Physiological Chemistry*" (Hawk), 5th Ed., page 539.)

β-Hydroxybutyric Acid.

A process of isolating and purifying β-hydroxybutyric acid, in the form of calcium-zinc hydroxybutyrate (a new double salt of calcium and zinc) has been reported by Shaffer and Marriott.[1] The oxidation method for the quantitative estimation of β-hydroxybutyric acid, suggested by Shaffer and given on page 407 of Vol. VII, is a very satisfactory one. The method has been used with success by Gorslin and Cooke,[2] Mondschein,[3] and others. In a re-examination of the method, called forth by the criticism of Embden and Schmitz,[4] Shaffer and Marriott have shown that the process yields 90% of the theoretical values. The results obtained by the method must therefore be corrected by adding one-ninth of the amount found. For use in blood analysis, a slight modification of the above method has been suggested by Marriott.[5]

The *extraction* method suggested by Black[6] is also very satisfactory for the quantitative determination of β-hydroxybutyric acid. The process is as follows: Take 50 c.c. of the urine under examination and make faintly alkaline with sodium carbonate; evaporate to one-third the original volume. Further concentrate to about 10 c.c. on a water-bath, cool the residue, acidify it with a few drops of concentrated hydrochloric acid, and add plaster of Paris to form a thick paste. Permit the mixture to stand until it begins to "set," then break it up with a stout glass rod having a blunt end, and reduce the material to the consistency of a fairly dry coarse meal. Transfer the meal to a Soxhlet apparatus, and extract with ether for 2 hours. At the end of this period evaporate the ether-extract, either spontaneously or in an air current. Dissolve the residue in water, add a little bone-black if necessary, filter until a clear solution is obtained, and make up the filtrate to a known volume (25 c.c. or less) with water. The amount of β-hydroxybutyric acid should then be determined by means of the polarimeter.

Black has also proposed a test, for the detection of β-hydroxybutyric acid, which is very satisfactory. (For details of this test consult Black's original article (*loc. cit.*), or "*Practical Physiological Chemistry*" (Hawk), 5th Ed., p. 441.)

Bile Acids.

A very satisfactory and simple test to detect bile acids is that of Hay, which is based upon the principle that bile acids have the property of reducing the surface tension of fluids in which they are contained. The test is as

[1] *J. Biol. Chem.*, 1913, 16, 265.
[2] *J. Biol. Chem.*, 1912, 10, 291.
[3] *Biochem. Zeit.*, 1912, 42, 95.
[4] *Handb. d. biochem. Arbeitsmeth.*, 3, 934.
[5] *J. Biol. Chem.*, 1913, 16, 293.
[6] *J. Biol. Chem.*, 5, 207.

follows: Cool about 10 c.c. of urine in a test-tube to 17° C. or lower, and sprinkle a little finely pulverised sulphur upon the surface of the fluid. The presence of bile acids is indicated if the sulphur sinks to the bottom of the liquid; the rapidity with which the sulphur sinks depending upon the amount of bile acids present in the urine. The test is said to show the presence of bile acids when the latter are present in the ratio of 1:120,000. It is claimed by some that this test does not differentiate between bile acids and bile pigments. The modification of the Pettenkofer test (see Vol. VII, p. 420), as proposed by v. Udransky, is also very satisfactory. This modification is as follows: To 5 c.c. of urine in a test-tube, add 3-4 drops of a very dilute (1:1,000) aqueous solution of furfural. Place the thumb over the top of the tube, and shake until a thick foam is formed. By means of a small pipette, add 2-3 drops of concentrated sulphuric acid to the foam, and observe the *dark pink* colouration produced.

A method for the preparation of the unconjugated acid of ox bile has been proposed by Schryver.[1]

The most recent analyses of bile are those reported by Menzies,[2] and Rosenbloom.[3] In each case the bile was obtained from a fistula and the composition (parts per 1,000 by weight) was as follows:

Constituent	Observer	
	Menzies	Rosenbloom
Bile salts	4.2	10.1
Mucin and pigments	9.3	4.86
Cholesterol	0.94	2.61[5]
Fat	2.98[4]	6.85
Soaps		2.6
Lecithin	not determined	6.42
Total solids	22.5	29.8
Inorganic	5.8	9.2
Water	974.5	970.2
Fatty acids	not determined	1.2

Bile Pigments.

The following process to estimate the bile pigments has been suggested by Czylhary, Fuchs and v. Furth:[6] 5 c.c. of bile are heated with 5 c.c. of 10% sodium hydroxide for 30 minutes on a steam-bath under a reflux; this treatment changing the bilirubin to biliverdin. The solution is mixed with 30 c.c. of 95% alcohol and the precipitate formed is removed by filtering. The filtrate is cleared by adding a few drops of hydrochloric acid and the green solution is compared in a colourimeter with a 0.02% alcoholic solution of Schuchardt's biliverdin.

[1] *J. Physiol.*, 44, 275.
[2] *Biochem. J.*, 1912, 6, 210.
[3] *J. Biol. Chem.*, 1913, 14, 241.
[4] Includes lecithin and fatty acids.
[5] Contained a trace of cholesterol esters.
[6] *Biochem. Zeit.*, 49, 120.

Apart from the tests for bile pigments mentioned in Vol. VII, p. 425, the following tests are often employed:

Salkowski-Schipper's Test.—Neutralise the acidity of 10 c.c. of the urine under examination with a few drops of a dilute solution of sodium carbonate, and add 5 drops of a 20% solution of sodium carbonate and 10 drops of a 20% solution of calcium chloride. Filter off the resultant precipitate, upon a hardened filter paper, and wash it with water. Remove the precipitate to a small porcelain dish, add 3 c.c. of an acid-alcohol mixture (made by adding 5 c.c. of concentrated hydrochloric acid to 95 c.c. of 96% alcohol) and a few drops of a dilute solution of sodium nitrate, and heat. The production of a green colour indicates the presence of bile pigments.

Bonanno's Test.—Place 5–10 c.c. of the urine under examination in a small porcelain evaporating dish and add a few drops of Bonanno's reagent (2 grm. of sodium nitrite in 100 c.c. of concentrated hydrochloric acid). If bile is present an emerald-green colour will develop. Bonanno says the test is not interfered with by any known normal or pathological urinary constituent.

Urinary Calculi.

Among the more recent analyses of urinary calculi are those of Kahn and Rosenbloom.[1] They analysed 24 calculi and showed 18 of them to contain over 60% of calcium oxalate; the 6 which contained less than 60% of calcium oxalate gave an average of 56% P_2O_5. All contained uric acid or urates, but only 3 of the 24 contained more than 10%. The authors claim that the shape, colour and consistency of a stone do not form a criterion of its composition. Two cystic calculi contained no calcium oxalate but 96.3% and 98.0% of uric acid respectively. The authors suggest acid treatment for oxalate calculi and alkali treatment for uric acid calculi.

A graphic scheme for use in the examination of urinary calculi, radically different from that given in Vol. VII, may be found in "*Practical Physiological Chemistry*" (Hawk), 5th Ed., p. 477.

Hydrochloric Acid.

Hydrochloric acid is manufactured in the human stomach by cells in the gastric mucosa. There are several theories as to the origin of this hydrochloric acid, *e.g.*, the *mass action* theory of Bunge, the *electrolytic dissociation* theory of Köppe, and the *phosphonuclease* theory of Bergeim.[2] The normal acidity of the human gastric juice has been placed at 0.2% HCl. However, recent investigations[3] have shown that the acidity of the gastric juice as secreted is probably about 0.4–0.5% HCl. Boldyreff (*loc. cit.*) claims that

[1] *J. Am. Med. Assn.*, 1913, 59, 2252.
[2] *Proc. Soc. Exp. Biol. Med.*, 1914, 12, 21.
[3] Babkin, "*Die äussere Sekretion der Verdauungsdrüsen*," Berlin, 1914; Boldyreff, *Quart. Jour. Exp. Physiol.*, 1914, 8, 1; Bergeim, Rehfuss and Hawk, *J. Biol. Chem.*, 1914, 19, 345; and others.

this acidity may be reduced to 0.2% by the regurgitation of alkaline juices from the intestine.

When the acidity of the gastric juice is increased to any extent above normal, a condition known as *hyperacidity* results; a decrease from normal is called *hypoacidity*. The volume of the gastric juice as well as its acid concentration may be increased by water ingestion.[1] Milk, and the extractives of meat, are also active gastric stimulants. A psychical stimulation may be brought about through the thought or sight of food. The hydrochloric acid of the gastric juice forms a medium in which the pepsin can most satisfactorily digest protein food. The acid acts also as a germicide, thus preventing putrefaction from taking place in the stomach, and possesses the power of inverting cane sugar. When protein food reaches the stomach it combines with the hydrochloric acid and forms so-called "combined hydrochloric acid," which is really a *protein salt* of hydrogen chloride and behaves differently from the *free* acid. In gastric analysis, the acid concentration may be determined by estimating the H^+ concentration, or by titrating with phenolphthaleïn as indicator for *total* acidity, and with Sahli's reagent (see Sahli's "*Diagnostic Methods*") for *free* acidity.

[1] Wills and Hawk, *J. Am. Chem. Soc.*, 1914, **36**, 158; Foster and Lambert, *J. Expt. Med.*, 1908, **10**, 820; Bergeim, Rehfuss and Hawk, *J. Biol. Chem.*, 1914, **19**, 345.

LACTIC ACID.

By W. A. DAVIS.

Qualitative Tests.—According to Neuberg[1] the statement frequently found in text-books that sarcolatic acid (d-lactic acid) does not give the iodoform reaction (compare Vol. VII, p. 435) is not correct as both d- and l-lactic acids respond to this test. Pyruvic acid, aldol, β-hydroxybutyric acid, quercitol and inositol also give a positive result under the same conditions, but in these cases the yellow precipitate formed, which has the characteristic odour of iodoform, may perhaps be a homologue of the latter.

British Pharmacopœia, 1914.—In the new pharmacopœia lactic acid (Acidum Lacticum) is defined as "an aqueous solution containing not less than 75% by weight of hydrogen lactate, $HC_3H_5O_3$, and not less than 100% by weight of lactide, $C_6H_8O_4$. Sp. gr. about 1.21." This definition is not in accord with the view generally held that the substance derived from the lactic acid during concentration is principally lactic anhydride and not lactide. The other requirements are as follows: "1 grm. diluted with 10 c.c. of water requires for neutralisation not less than 8.3 c.c. of $N/1$ solution of sodium hydroxide. After the further addition of 10 c.c. of the alkaline solution and boiling for 15 minutes not more than 8.6 c.c. of $N/1$ solution of sulphuric acid are required to neutralise the excess of alkali. Yields no characteristic reactions for calcium, iron, chlorides, citrates, oxalates, phosphates, sulphates or tartrates. *Lead limit* 10 parts per million. *Arsenic* limit 5 parts per million." The remaining tests are the same as given in Vol. VII, p. 446.

Estimation of Lactic Acid in Organic Tissues and Extracts in Presence of β-Hydroxybutyric Acid and Other Substances.—According to Mondschein[2] previous determinations of the lactic acid in flesh have been too low owing to the fact that about one-third of the acid is carried down with the coagulated albumin and escapes estimation. The lactic acid in a decoction of muscular fibre can be estimated with sufficient accuracy by direct titration using phenolphthaleïn as indicator, since other acid substances are not present in appreciable quantities under normal conditions. The portion retained by the coagulated albumin may be estimated by boiling the latter with solution of alkali, precipitating the albumin from the solution of albuminate by adding a saturated solution of sodium chloride and estimating the lactic acid in the filtrate by oxidation into acid permanganate solution (see below).

[1] *Biochem. Zeitschr.*, 1912, 43, 500.
[2] *Biochem. Zeitschr.*, 1912, 42, 91 and 105.

When β-hydroxybutyric acid is present as well as lactic acid Mondschein adopts the following process. Separate portions are taken for the analysis. In one of these the lactic acid is oxidised by potassium permanganate in 1% sulphuric acid by the method of Fürth and Charnass (see Vol. VII, p. 439), the products consisting of acetaldehyde from the lactic acid and a certain amount of acetone from the β-hydroxybutyric acid. The distillate is divided into two parts in one of which the absorption value for sulphurous acid is determined by Ripper's method of titration into iodine[1] whilst the second is freed from acetaldehyde, by boiling with alkali and hydrogen peroxide, beneath a reflux condenser, and is then again distilled and titrated according to Ripper's method. The difference between the two results is due to the acetaldehyde present in the first distillate and is calculated as *lactic acid*. The other portion of the original distillate is oxidised with 0.5% potassium dichromate solution in 4% sulphuric acid which oxidises the β-hydroxybutyric acid to acetone and a little of the lactic acid to acetaldehyde. The mixture is distilled, the second distillate freed from acetaldehyde as before and the acetone distilled and estimated volumetrically by means of iodine and thiosulphate; the acetone found is calculated as β-hydroxybutyric acid.

According to Oppenheimer[2] the method used by Mondschein of liberating the lactic acid from proteins by means of boiling 10% sodium hydroxide is open to several objections and he recommends precipitating the proteins with hydrochloric acid and mercuric chloride as proposed by Schenk. In comparative experiments with blood, muscle and yeast-juice it was found possible to recover, by the latter method over 96% of the lactic acid added to these fluids. Colloidal iron used as precipitant is not satisfactory as a loss of about 30% of the lactic acid occurs therewith.

Bellet[3] gives the following details of a method of estimating lactic acid in substances such as blood, urine, etc., based on its conversion into acetaldehyde by permanganate. The liquid is first freed from proteins by means of Patein and Dufau's reagent (an acid solution of mercuric nitrate) and is then neutralised and evaporated to a syrup; the latter is acidified with sulphuric acid, mixed with anhydrous sodium sulphate and sand, and extracted with ether in a Soxhlet apparatus. The ethereal extract is evaporated, the residue dissolved in water, transferred to a flask and diluted to 200 c.c. The flask is connected with a condenser the lower end of which passes into an absorption vessel; this in turn is connected with a second similar vessel and condenser. The absorption vessels contain a definite volume of a solution of silver nitrate (15 grm. silver nitrate, 150 grm. ammonia and 100 c.c. sodium hydroxide solution, the mixture being diluted to 500 c.c.). The liquid in the flask is made strongly acid with sulphuric acid, heated to boiling and a 1.5% solution of potassium permanganate added drop by drop at such a rate that each drop is decolourised before the next is intro-

[1] *Monatsh.* 1900, 21, 1079.
[2] *Zeit. physiol. Chem.*, 1914, 89, 39.
[3] *Bull. Soc. Chim.*, 1913, 13, 565.

duced; a current of air is kept passing through the apparatus so as to carry over the acetaldehyde as fast as it is formed. When the permanganate is no longer decolourised the solution is boiled for an additional 3 minutes, the contents of the receivers are mixed, filtered and the excess of silver is titrated. The presence of β-hydroxybutyric acid, succinic and oxalic acids does not interfere with the estimation of lactic acid by this method.

It is probable that the methods described above, depending upon the oxidation of lactic acid to acetaldehyde, a change which is difficult to regulate so that it shall take place quantitatively, are not so accurate as the method described in Vol. VII, depending on the separation of the zinc salt (Vol. VII, pp. 437 and 451). Wolf[1] has recently described experiments emphasising this; he shows that the estimation by the aid of the zinc salt in general is far more satisfactory than by oxidation methods. In concentrating the fluids for analysis, they should be evaporated *in vacuo* at as low a temperature as possible so as to avoid loss of the acid. The extraction from the concentrated solution by ether is best accomplished by absorbing the solution on blotting paper and extracting in a Soxhlet apparatus three or four times. Wolf, like Oppenheimer (*supra*), uses Schenk's method to remove the proteins.

For other methods of estimating latic acid see page 608.

Estimation of Lactic Acid in Urine.—Baragiola and Schuppli[2] describe a modification of Möslingers method (Vol. VII. p. 438), as the latter is likely to give unreliable results owing to the reduction of barium chloride by carbon, to the retention of lactic acid by the substances insoluble in alcohol and to the conversion of the lactic acid by malic acid into a compound in which the former is not estimated. The modified method is as follows: 25 c.c. of the urine, mixed with 25 c.c. of water, are distilled with steam until 200 c.c. of distillate have been collected; 5 c.c. of 10% barium chloride are added to the distillate and the latter neutralised with saturated barium hydroxide. In case lactic anhydride is present, an excess of barium hydroxide may be added, the solution heated over the water-bath and neutralised with hydrochloric acid using azolitmin as indicator. The neutral solution is evaporated to 15 c.c., transferred to a 100 c.c. cylinder, diluted with water to 25 c.c. and 95% alcohol added drop by drop, shaking well. The mixture is diluted to 100 c.c. with alcohol, filtered after 24 hours, and 75 c.c. of the filtrate mixed with 25 c.c. of 5% solution of sodium sulphate. The barium sulphate is filtered off and 75 c.c. of the filtrate are evaporated to dryness. The residue is incinerated, the ash dissolved in water and the boiling solution titrated with $N/10$ acid.

[1] *J. Physiol.*, 1914, **48**, 341.
    Zeitsch. Nahr. Genussm., 1914, **27**, 841.

CYANOGEN AND ITS DERIVATIVES.

By HERBERT PHILIPP.

Spectroscopic Detection of Cyanogen.—W. Grotrian and C. Rung[1] report on the so-called "cyanogen bands." A Schönherr's arc which was filled with nitrogen, that was also mixed with other products (CO_2, etc.) between copper, iron, platinum, aluminium and carbon electrodes, proved that the so-called cyanogen bands were due to nitrogen and that cyanogen cannot be detected spectroscopically.

Hydrocyanic Acid and Simple Cyanides.

Sodium cyanide, NaCN, has a solubility of 51.7 grm. in 100 c.c. water and the salt melts at 560°.

Gold cyanide compounds have been recommended for therapeutical use in the treatment of tuberculosis and syphilis, generally together with organic bases to prevent the reduction of the gold cyanide. Its therapeutical value is, however, disputed by some authorities.

Detection of Hydrocyanic Acid and Cyanides.—O. L. Barnely[2] proposes a new method of detecting cyanides in the presence of ferro- and ferricyanides and thiocyanates which depends on the solubility of cupric sulphide in alkali cyanide solutions. When hydrogen sulphide is passed into a dilute ammoniacal cupric solution a precipitate of cupric sulphide is formed, or a deep blue to brownish-black colouration is imparted to the solution. The addition of an alkali cyanide clears this suspension or coloured solution; ferro- as also ferricyanides and thiocyanates do not disturb the reaction. The test is carried out with quite dilute copper solutions, an ammoniacal solution of 1.25 grm. $CuSO_4,5H_2O$ to the litre being used. Each c.c of this solution is equivalent to about 0.000473 grm. of hydrocyanic acid. The theoretical action is

$$2(CuSO_4 \cdot 4NH_3) + 7KCN + H_2O = 2K_2Cu(CN)_3 + KCNO + K_2SO_4 + (NH_4)_2SO_4 + 6NH_4OH$$
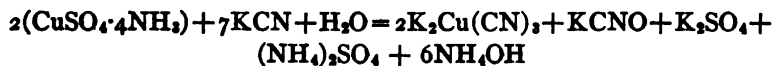
The approximate amount of alkali cyanide can be estimated by this method.

C. Pertusi and E. Gastaldi[3] describe a method to detect hydrocyanic acid and cyanides. A few drops of a 3% copper acetate solution are put into a test-tube to which is added 1 c.c. of a 10% disodium phosphate solution and 4

[1] *Phys. Zeit.,* 1914, 15, 545.
[2] *J. Amer. Chem. Soc.,* 1914, 36, 1092.
[3] *Chem. Zeit.,* 1913, 37, 609.

drops of a saturated benzidine acetate solution. To this the solution to be tested is added drop by drop. It is necessary to add the solution to the reagents and not *vice versa* as the cyanogen liberated through the copper salt from the cyanide only reacts instantaneously *in statu nascendi*. By using these precautions, a distinct blue violet colouration is obtained by adding 6 drops of a solution containing 0.000027 grm. HCN per c.c.

If the solution to be tested contains a large amount of sulphocyanide action will take place in the absence of cyanides and in this case the method must be carried out as follows: The material to be tested must be, as is generally the case with acids, boiled with a solution of sodium carbonate. The filtered solution is placed in a gas wash-bottle and a current of carbon dioxide, washed by means of a solution of sodium carbonate, is passed through. The carbonic acid being a stronger acid expels the hydrocyanic acid from the alkali cyanide.

Iron, cobalt, manganese, *i.e.*, such metals which in alkali solution with cyanides form double salts, must not be present.

This last described method is especially delicate. In 10 c.c. of liquid 0.000007 grm. hydrocyanic acid are distinctly traceable, quantities which do not show by the Prussian blue reaction.

This test is also recommended to detect nitrogen in organic compounds after conversion into cyanide by metallic sodium, further for the examination of gas, contaminated air, etc.

Estimation of Hydrocyanic Acid and Cyanides.—A method of estimating hydrocyanic acid and the alkali cyanides has been worked out by G. E. F. Lundell and J. A. Bridgman.[1]

This new method is based upon the titration of an ammoniacal hydrocyanic acid solution with nickel ammonium sulphate solution in the presence of dimethylglyoxime. The characteristic red colouration of the nickel dimethylglyoxime appears only after all of the cyanide is bound as the nickel double salt. The changes involved are as follows:

$$NiSO_4 + 4KCN = K_2Ni(CN)_4 + K_2SO_4$$
$$NiSO_4 + 2C_4H_8N_2O_2 = Ni(C_4H_7N_2O_2) + H_2SO_4$$
$$H_2SO_4 + 2NH_4OH = (NH_4)_2SO_4 + H_2O$$
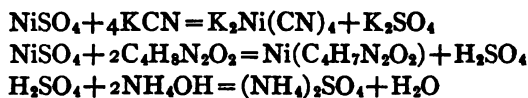
No permanent red precipitate of nickel dimethylglyoxime is formed until all of the cyanide has been used up according to the first equation. The ammoniacal cyanide solution is used since free sulphuric acid hinders the precipitation of nickel dimethylglyoxime.

The reagents required consist of a solution of 15.3 grm. of nickel ammonium sulphate in 1000 c.c. water to which is added 2 c.c. of concentrated sulphuric acid (50 c.c. shall be equivalent to 1 grm. KCN) and a solution of 8.9 grm. of dimethylglyoxime in 1000 c.c. 95% alcohol.

To carry out the estimation 5 grm. of the sample are dissolved in water and

[1] *J. Ind. and Eng. Chem.*, 1914, **6**, 554.

diluted to exactly 500 c.c. 50 c.c. of this solution are diluted with an equal volume of water treated with 1 c.c. of ammonium hydroxide and 0.5 c.c. of the dimethylglyoxime solution and then titrated with the nickel solution until a permanent red precipitate is produced. The results agree well with other methods. The presence of double cyanides, except potassium zinc cyanide, does not disturb the method.

A new and excellent method of estimating halogens in alkali cyanides is described by Polstorff and Meyer.[1] Exactly 0.6 grm. of substance is dissolved in 100 c.c. distilled water. If it is necessary to make the solution alkaline, precautions must be taken to ensure that the sodium or potassium hydroxide used is absolutely free from halogens. 20 to 30 drops of commercial formaldehyde solution (35%) are added to the alkaline solution, which is allowed to stand for 15 minutes and then acidified carefully with 30% nitric acid (5 c.c. usually suffices). The halogen is estimated in the solution by the Volhard Method described in Vol. VII, p. 553.

Double Cyanides.

Sodium Zinc Cyanide.—N. Herz[2] reports that sodium zinc cyanide, $Na_2Zn(CN)_4,3H_2O$, crystallises from pure solutions of sodium zinc cyanide which are free from less soluble salts and from excessive amounts of free alkali. One part of this hydrated salt is soluble in 0.47 part water at 15°. The crystals are orthorhombic, simple in form, either flat diamonds or pseudo-hexagonal plates. They are brilliant when removed from the solution but effloresce rapidly, becoming dull and chalky. The dry salt is quite stable in air, being completely soluble in water after several days' exposure. Even after complete dehydration at 105° there was very little evidence of decomposition. •

Compounds of Cyanogen and Iron.

On page 503, Vol. VII, line 2, $Fe^{III}\begin{cases} (CN)_3 \\ (CN)_3 \end{cases} =$

should read $Fe^{III}\begin{cases} (CN)_3 \\ (CN)_3 \end{cases} =$.

In 1912 I. Guareschi discovered that a fuchsine solution decolourised with sulphurous acid was the best reagent for detecting bromine.[3] He later applied this method to detect bromides in the presence of ferro- and ferri-cyanides.[4] The fuchsine solution is prepared by dissolving 1 grm. fuchsine hydrochloride in 1000 c.c. distilled water, to which is added, whilst stirring, 8 c.c. of a saturated sodium bisulphite solution and about 10 c.c. hydrochloric acid (1.19 sp. gr.). Starch-free filter paper is saturated with this solution for

[1] Zeit. anal. Chem., 1912, 51, 601.
[2] J. Amer. Chem. Soc., 1914, 36, 912.
[3] Zeit. f. anal. Chem., 1913, 52, 538.
[4] Atti R. Accad. Sci. Torino, 1913, 49, 15.

use in detecting bromine, which forms a blue colour when it comes into contact with this paper or the solution as prepared above.   To carry out the test the material to be tested is usually treated dry or in solution with a 50% solution of chromic acid, gently heated with a piece of the test paper in the neck of the flask, when the presence of bromides can be detected by the blue colouration of the paper.

0.001 grm. potassium bromide can be detected in 1 grm. of potassium ferro- or ferricyanide.   Smaller amounts can be detected by treating the powdered material with a 25% chromic acid solution.   This method can also be used to detect bromides in Prussian blue.

Estimation of Ferrocyanides.—L. L. de Koninck and N. Joastart[1] have suggested the use of alkali bromate for the titration of ferrocyanides instead of that of permanganate solution.   The advantage claimed is that no foreign metals enter the solution which might be precipitated by using the solution for further analysis.   The titration takes place in acid solution and iron alum is used as indicator.

Estimation of Hydroferricyanic Acid in the Presence of Ferric and Cyanogen Ions.—E. Müller and F. Seidel[2] have worked out a satisfactory method of estimating ferricyanides in the presence of ferric salts and cyanides. The method depends on the introduction of potassium fluoride into the solution, which has the effect of preventing the liberation of iodine from potassium iodide in the presence of ferric salts whilst not interfering with its liberation from potassium iodide by ferricyanide salts.

The ferricyanide solution containing a ferric salt is treated with an excess of potassium fluoride solution (0.386 grm. per c.c.) in relation to the quantity of ferric salt present, then with potassium iodide and finally with some zinc sulphate solution.   It is necessary to add the zinc sulphate solution last as otherwise zinc ferricyanide is formed which liberates iodine from the potassium iodide very slowly.   The liberated iodine is titrated with sodium thiosulphate as described in Vol. VII, p. 528.

If cyanogen ions are present it is necessary to add a few drops of sulphuric acid and to expel the hydrocyanic acid by a current of carbon dioxide gas, which is first passed through a potassium permanganate solution so as to be sure that it is free from reducing gases.   If the hydrogen cyanide is passed through a wash bottle containing silver nitrate solution it can be estimated quantitatively at the same time.

Carbonyl Ferrocyanides.

H. E. Williams[3] states that compounds of hydrocarbonyl-ferrocyanic acid, $H_2FeFe(CN)_5CO$, exist in the mother liquor resulting from the working up of cyanogen mud, better known as spent purifying mass of

[1] *Chem. Zeit.,* 1914, **38**, 1084.
[2] *Zeit. anal. Chem.,* 1914, **53**, 416.
[3] *Proc. Chem. Soc.,* 1913, **29**, 10.

gas works. The carbonyl-ferrocyanic radical is recovered by precipitating with ferric salts, boiling the precipitate with lime and precipitating the ferrocyanide present as calcium ammonium ferrocyanide. The filtrate is boiled with lime and the salt allowed to crystallise. The salts of the alkali and alkali earth metals, including lithium and magnesium, are very soluble and several are deliquescent. The salts of the heavy metals are for the most part insoluble in water.· Lead, chromic, stannic, and aluminium salts produce no precipitate.

Thiocyanates, Sulphocyanides.

Guareschi's test (see page 587) can also be used to detect bromides in the presence of sulphocyanides by treating either the substance or a concentrated solution of the same with chromic acid. More precautions have to be taken in this determination than described under ferrocyanides. The process is carried out as follows: A mixture of 0.5 grm. potassium sulphocyanide and 0.001 grm. potassium bromide are carefully treated with 10 c.c. of a 50% chromic acid solution. When the vigourous action has subsided the mixture is gently heated until it boils, care being taken that the long-necked flask does not become too hot from the steam. If now a gentle stream of air is passed through the flask the decolourised fuchsine test paper (see page 588) becomes blue. This method will detect the presence of 0.0003 grm. potassium bromide in 0.5 grm. potassium sulphocyanide.

In detecting bromides in ammonium sulphocyanide it is best to treat the substance with an excess of 5 or 10% chromic acid solution.

If potassium permanganate is used instead of chromic acid for oxidising, errors are liable to ensue as the formed cyanate salt easily decomposes, producing ammonia according to the following equation:

$$HCNO + H_2O = CO_2 + NH_3$$
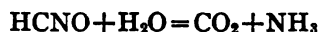
The liberated ammonia gives a light red colour to the fuchsine reagent which might easily cover the blue produced by the bromine. This method can be used in the presence of iodides and chlorides.

Cyanamide.

A. P. Lidow[1] states that the commercial cyanamide salts lose their nitrogen by storing in the atmosphere. Calcium cyanamide is claimed to lose up to 32.62%, whilst sodium cyanamide loses up to 33.8% of the original nitrogen. The loss of nitrogen is not only connected with the escape of ammonia, but also due to the formation of α oxane salts.

The small quantities of unconverted carbide left in the commercial calcium cyanamide (lime nitrogen) can become dangerous on storing in a

[1] *Chem. Zeit.*, 1914, **38**, 574.

closed space, as was the case last January[1] when 600 sacks on board a ship in Trieste harbor evolved enough acetylene to create a violent explosion. This calcium cyanamide was packed in sacks, but it has been ordered in future to pack this material in iron drums for transportation.

On page 558, Vol. VII, a method is described for the estimation of calcium cyanamide which has been modified by G. Grube and J. Krüger[2] who proceed as follows: An alkali or alkali earth cyanamide solution containing 60 grm. cyanamide to the litre is used. 10 c.c. of this solution are made up to 100 c.c. and of this dilute solution 10 c.c. are placed in a 500 c.c. measuring flask which contains 300 c.c. of water acidified with 1 c.c. $2N$ nitric acid. To this solution are added 50 c.c. of $N/10$ silver nitrate solution and so much 2% ammonium hydroxide solution that a piece of litmus paper placed in the solution just turns blue. The flask is then filled to the mark, vigorously shaken until the precipitate gathers together; 250 c.c. of the filtered solution are titrated with $N/10$ ammonium thiocyanate to estimate the excess silver used. It was found that a large excess of ammonium hydroxide or ammonium salts gave erroneous results on account of the solubility of silver cyanamide in such solutions.

[1] J. Frank. Inst., 1914, 178, 247.
[2] Zeit. angew. Chem., 1914, 27, 326.

ENZYMES.

By E. FRANKLAND ARMSTRONG.

For the investigation of problems concerning plant oxydases (see Vol. VIII, p. 12) it is probable that the best results will be gained by using several oxydase reagents to find that most suited for the particular case. One of the most satisfactory reagents is benzidine, used either in $\frac{1}{2}\%$ solution in 50% ethyl alcohol or as a saturated solution in 1 or 2% sodium chloride (compare Keeble and Armstrong).[1] Blue or violet brown colourations or precipitates are obtained when the reaction is positive, owing to the formation of *meri*-quinonoid salts of dipheno-quinonediimine with benzidine.

According to Bach,[2] guaiacol is the most suitable substance for testing the sensitiveness of the peroxydase reaction on account of its relative resistance to hydrogen peroxide in the absence of a catalyst. He uses a 0.1% solution of guaiacol in water.

Chodat[3] has shown that a remarkable range of coloured compounds is produced when a vegetable oxydase acts on *p*-cresol in presence of an amino-acid, polypeptide or peptone, according to the nature of the amino-compound.

Bunzel[4] describes a simplified apparatus for measuring the oxydase in liquids, in which the plant juice and a solution of an oxidisable substance are mixed under known conditions and the change in volume due to the oxygen absorbed in the action is measured.

Kober[5] applies the estimation of suspensoids by means of the nephelometer to the study of enzymes. In the case of the nucleases, undigested nucleic acids are precipitated by a 0.2% solution of egg albumin faintly acidified with acetic acid.

[1] *Proc. Roy. Soc.*, 1913, **87B**, 125.
[2] *Ber.*, 1914, **47**, 2122.
[3] *Arch. Sci. phys. nat.*, 1912, [iv], **33**, 70.
[4] *Chem. Soc. Abstracts*, 1913, II, 508.
[5] *J. Amer. Chem. Soc.*, 1914, **36**, 1304.

PROTEINS.

By S. B. SCHRYVER, Ph.D., D. Sc.

Estimation of Aspartic and Glutamic Acids in the Products of Protein Hydrolysis.—F. W. Foreman[1] has described the following method: If calcium hydroxide is added to the hydrolysis mixture (after evaporating off the greater part of the hydrochloric acid used *in vacuo*), and then alcohol, the calcium salts of glutamic and aspartic acids are precipitated quantitatively together with other substances. If the free acids are regenerated from the calcium salts, they are obtained in a crystalline form and can be separated from pyrrolidinecarboxylic acid and other substances, which up to the present have not been identified, by treatment with cold glacial acetic acid in which the aspartic and glutamic acids are insoluble. The proportion of the two acids can be determined by estimating the carbon in the mixture or by separating them by means of the copper salts. The pyrrolidinecarboxylic acid can be estimated in the acetic acid extract by determining the amino-nitrogen in weighed portions before and after hydrolysis with hydrochloric acid, an operation which causes its conversion into glutamic acid. It appears that under the conditions employed up to the present, some glutamic acid is converted into pyrrolidinecarboxylic acid during the operations, but it is believed that a modification of the conditions will be found under which this change will be avoided.

[1] *Biochem. J.*, 1914, **8**, 463.

VEGETABLE PROTEINS—FLOUR

By E. FRANKLAND ARMSTRONG.

Modern milling practice—in particular the processes of improving and conditioning—has directed attention to the testing of wheat and flour for moisture, phosphate, sulphate, nitrite, etc.

The degree of bolting or dressing of flour is best determined by eye. According to Lindet[1] the cellulose content forms an accurate basis for evaluation.[2]

Frequent estimations of moisture are now a matter of necessity in scientific milling and quick and reliable methods of testing are required. The methods based on heating the grain in a mineral oil in suitable apparatus and measuring the water which distils over take about half an hour. In any method of determining moisture the influence of fluctuations of a few degrees in the temperature to which the grain or flour is heated must not be overlooked. (See in this connection S. Lovatt, *J. Ind. Eng. Chem.*, Jan., 1910.)

The recent extension of the use of processes of conditioning and bleaching in milling brings the search for the presence of improvers in flour within the analyst's range. The commonest of these are acid calcium phosphate with or without acid ammonium phosphate. These are often sold mixed with flour. The presence of calcium sulphate in any considerable proportion is to be regarded as an adulteration. Another type of improver consists of potassium persulphate, $K_2S_2O_8$. "*Salox*" is stated to contain 2% of this salt mixed with flour; only 1 ounce is said to be required for a sack of flour, so that its detection is practically impossible.

Treatment of flour by spraying with soluble improvers—generally soluble phosphates—leads to an increase of phosphate in the flour. This can be detected by analysis only if a typical flour of similar origin is available for comparison. As flours from the same locality vary a good deal from season to season in their content of phosphate, no empirical standard can be given.

It is impossible to determine sulphates in the ash of flours as the acid phosphates present decompose sulphates on ignition. The method proposed by Cripps and Wright[3] is to shake 100 grm. of flour with 1,000 c.c. of 1% acetic acid for 1 hour, then take 500 c.c., boil this with hydrochloric

[1] *Bull. Soc. Chim.*, 1914, 15, 384.
[2] *Chem. Soc. abstracts*, 1914, II, 500.
[3] *Analyst*, 1914, 39, 429.

acid, nearly neutralise, precipitate the proteins with Almen's tannin reagent, filter and estimate the sulphate in the filtrate.

In various flours examined the amount varied from 0.0069 to 0.0084% of SO_3.

R. T. Thomson[1] heats about 20 grm. of flour with dilute hydrochloric acid until the starch is liquefied, and determines sulphates in the filtrate by means of barium chloride. He states that first-grade flour contains 0.01 to 0.013% of SO_3, whilst third grade contains 0.061%.

According to R. T. Thomson ordinary wheaten flour is neutral to litmus and methyl-red, alkaline to methyl-orange, and acid to phenolphthalein. During milling, nitrite equivalent to about 0.35 part of $NaNO_2$ per million may be absorbed from the atmosphere.

According to Thatcher and Koch[2] a quantitative extraction of diastase from flour can be made by shaking with water at 0° from 1 to 2 hours. The liquid is filtered cold and the diastatic power estimated immediately.

Addendum to Vol. VII, p. 93.—More recent analyses by Smetham of a great variety of vegetable feeding stuffs are given in "*The Analyst*," 1914, **39**, 481; *J. Soc. Chem. Ind.*, 1914, p. 1107.

ERRATUM IN VOL. VII.

Page 104, line 9, "122.9°" should read "−122.9°."

[1] *Analyst*, 1914, **39**, 519.
[2] *J. Amer. Chem. Soc.*, 1914, **36**, 759.

PROTEINS OF MILK.

BY L. L. VAN SLYKE.

Method of Preparing Ash-free Casein.—The method described on pages 116–117, Vol. VIII, is modified as follows by Van Slyke and Bosworth:[1] After the casein has been precipitated and redissolved four or more times, the final solution in dilute ammonia is treated with 10 c.c. of strong ammonia and then with 20 c.c. of saturated solution of ammonium oxalate. The mixture is allowed to stand 12 hours or more. The precipitated calcium oxalate is removed by centrifugal force and subsequently by filtering through a double thickness of filter paper. The filtered solution is next treated with dilute hydrochloric acid (10 c.c. acid, sp. gr. 1.20, diluted to 1 litre) until the casein is precipitated. The precipitate is washed with distilled water until free from chlorides and is then placed on a hardened filter paper in a Buchner funnel, as much water as possible being removed by suction. The mass is next transferred to a large mortar and triturated thoroughly with 95% alcohol and, after its removal by suction, again triturated with absolute alcohol; after filtering by suction, the mass is twice treated with ether, which is removed each time by suction. The material is then spread out in a thin layer and allowed to dry in a warm place for 12 hours. It is finally ground in a mortar so as to pass a 40-mesh sieve and dried for 2 days over sulphuric acid under diminished pressure. Such preparations were found to contain less than 0.10% ash. The phosphorus content of such preparations is about 0.70% instead of 0.85%, the amount obtained with casein made under ordinary conditions without the removal of the calcium.[2]

Compounds of Casein and Paracasein with Bases.—The existence of caseinates and paracaseinates in addition to those containing 1.50% and 2.50% of CaO has been shown.[3] Compounds of casein with K, Na, NH₄, Mg, Ca, Sr and Ba have been prepared; the general results are shown in the following table:

Basic elements	Mono-basic compounds of casein; 100 grm. of casein combine with, grm.	Di-basic compounds of casein; 100 grm. of casein combine with, grm.
NH₄	0.20	....
Na	0.26	....
K	0.44	....
Mg	0.13	0.24
Ca	0.22	0.44
Sr	0.48	0.96
Ba	0.76	1.51

[1] *J. Biol. Chem.*, 1913, **14**, 203.
[2] Bosworth and Van Slyke, *J. Biol. Chem.*, 1914, **19**, 67.
[3] Van Slyke and Bosworth, *J. Biol. Chem.*, 1913, **14**, 211, and Van Slyke and Winter, *ibid.*, 1914, **17**, 287–291.

Corresponding paracaseinates have been prepared, but they contain twice the amount of base contained in the caseinates. Mono-basic caseinates and paracaseinates of ammonium, sodium and potassium are soluble in water, but those of magnesium, calcium, strontium and barium are insoluble in water but are soluble in warm 5% solutions of sodium, ammonium and potassium chlorides. The solubility is due to an exchange of bases; the action, for example, between mono-calcium caseinate and sodium chloride results in the formation of the soluble sodium caseinate and calcium chloride. The action is reversible. Di-basic caseinates are soluble in water and are easily precipitated by soluble salts of calcium, strontium, barium, etc.

Molecular Weights and Valency of Casein and Paracasein.—On the basis of the analytical results obtained in studying the composition of the mono-basic and di-basic caseinates and paracaseinates, together with other facts, Van Slyke and Bosworth[1] have concluded that the molecular weight of casein is 8888 and that of paracasein 4444, and also that the valency of the protein molecule in basic caseinates is 8, in basic paracaseinates, 4.

Estimation of Casein.—Richmond's method for the estimation of total proteins in milk by means of formaldehyde[2] has been adapted by Walker[3] to estimate casein in milk. To 10 c.c. of milk is added about 1 c.c. of a 1% solution of phenolphthaleïn and then $N/9$ solution of sodium hydroxide is added with constant stirring until a fairly deep pink colour appears and remains. No account is kept of the amount of alkali thus used. Then about 2 c.c. of neutral 40% formaldehyde solution is added, when the pink colour disappears. The reading of the burette is now recorded and alkali is again added with stirring until the same intensity of colour results as in the first titration. The reading of the burette is again taken and the difference between the two readings gives the number of c.c. of alkali used in the second titration, and this figure multiplied by 1.63 gives the percentage of casein in the milk.

If a sample of 16.3 c.c. of milk is used, the reading of the burette gives the percentage of casein directly without multiplying by the factor 1.63. Solution of $N/10$ alkali can be used provided a 9 c.c. sample of milk is taken for the estimation, the final result being multiplied by 1.63.

The formaldehyde solution is kept neutral by adding a few drops of phenolphthaleïn to the formaldehyde solution in the bottle and then alkali until a pink colour remains.

The results obtained show excellent agreement with the A. O. A. C. method.

[1] *J. Biol. Chem.*, 1913, **14**, 227 and 231.
[2] *Analyst*, 1911, **36**, 9.
[3] *J. Ind. Eng. Chem.*, 1914, **6**, 131.

MILK PRODUCTS.

BY CECIL REVIS AND E. RICHARDS BOLTON.

Regulations in England, Local Government Board, Oct., 1912 (cf. Vol. VIII, p. 184).—Preserved cream is defined as cream having not less than 35% of milk fat by weight, and to which has been added (1) boric acid, borax or a mixture of these substances, or (2) hydrogen peroxide.

No thickening substance is to be added to cream, whether preserved or not.

No other preservative than those specified above may be used.

Cane sugar and beet sugar are not regarded as either preservatives or thickening agents. Calcium saccharate is forbidden.

All preserved cream must be labelled in accordance with the nature of the preservative.

The above regulations have led to considerable trouble since, though preservatives are allowed, the maximum quantity to be used is not specified, and it is left to individuals to settle what amount may be considered prejudicial to the public health.

In many cases the maximum has been placed at 0.25% calculated as boric acid (H_3BO_3), but this is not really sufficient in warm weather for trade purposes, and 0.35% at least should be allowed.

Regulations as to Preservatives in Food made by Canadian Order-in-Council, April 4, 1914.—The following preservatives are allowed in the quantities shown, unless satisfactory evidence of harmfulness shall be forthcoming. Only one preservative of any kind is allowed in any kind of food product, or in a mixture of two or three kinds of food:

(1) Boric acid. Limit, 1 part in 400 for cream, 1 part in 200 for butter and other foods.

(2) Benzoic acid (sodium benzoate). Limit, 1 part in 1,000 parts.

(3) Salicylic acid. Limit, 1 part in 5,000 parts.

(4) Sulphurous acid (bisulphite of soda, potash or lime). Limit, 1 part in 2,000 for solid foods.

(5) Saccharin. Limit, 1 part in 700 parts for solid foods.

The following preservatives are completely prohibited: formaldehyde (formalin), β-naphthol, abrastol (asaprol) hydrofluoric acid, fluorides, fluoborates, fluo-silicates, or other fluorine compounds.

Estimation of Sugars in Condensed Milk (cf. Vol. VIII, p. 213).— A careful investigation has recently been made by Revis and Payne[1] into the inversion factor to be used when acid mercuric nitrate is employed as

[1] *Analyst*, 1914, 39, 476.

coagulant and inversion agent for condensed milk. Not only does acid mercuric nitrate immediately commence to invert cane sugar but, as Richmond has shown, the precipitation of protein is not complete, the small quantity left having quite an appreciable effect on the readings. The new factor proposed by them obviates these sources of error.

In order to avoid the use of special measuring vessels graduated at novel temperatures, which are not likely to be in the ordinary laboratory, they have adopted the following procedure:

65.085 grm. of the condensed milk are weighed out and washed into a graduated flask containing 250 true c.c. adjusted at 15° C. (the ordinary standard Charlottenburg flask), with sufficient water to bring the volume to about 220 c.c. This is then heated in boiling water for 5–10 minutes, or else allowed to stand over night. In either case, the volume is eventually made up to the mark at 15° C. After well mixing, 100 c.c. are measured out into a long stoppered cylinder with a 50 c.c. pipette (50 c.c. = 50 grm. of water at 15° C.), 10 c.c (true c.c. at 15° C.) of acid mercuric nitrate added and the whole violently shaken for 30 seconds till the curd is in the finest possible state of division. The cylinder is allowed to stand for 5 minutes at 15° C. and the contents then filtered through a dry filter. As soon as sufficient for the direct reading has passed, its temperature is raised to 20° and the tube filled and the reading taken at that temperature. 25 c.c. of the remainder of the filtrate are placed in a 50 c.c. stoppered flask, counterpoised, and then heated in boiling water for 8 minutes, cooled, readjusted to the original weight, filtered, if necessary, and the invert reading taken as near 20° as possible.

The readings are corrected for the increase in volume due to the added mercuric nitrate less the volume of the fat (F) and protein (P) precipitated.

This correction (C) will be,

$$C = 10 - \left\{ \frac{(F \times 1.11) + (P \times 0.82)}{100} \times 26.034 \right\}$$

and the readings must be multiplied by (100 + C) to give the correct results for the normal weight. The following formulae then give the percentage of sugars.

$$\text{Cane sugar } \% = \frac{(D - I)100}{R_c - \frac{T}{2}} = S$$

$$\text{Lactose (anhydrous) } \% = (D - S) \times \frac{R_L}{26.034} \times 100$$

there D = corrected direct reading.

I = corrected invert reading.

$\left. \begin{array}{l} R_c = \\ R_L = \end{array} \right\}$ the inversion and lactose factors.

T = temperature of invert reading.

It is assumed that a Schmidt and Haensch quartz wedge compensating polarimeter is used, reading in saccharose units, and so graduated that when 26.048 grm. of saccharose are dissolved in 100 Mohr c.c. at 17.5° C. and polarised at 17.5° C. the reading is 100 scale divisions.

The change in the above formulæ of 26.048 to 26.034 is necessitated by the analytical procedure adopted.

As the result of many careful experiments the following values were obtained for R_c and R_L at sugar concentrations such as will be realised in dealing with condensed milk:

$$R_c = 141.71$$
$$R_L = 0.3086$$

Aldehyde Figure for Cream (Vol. VIII, p. 187).—Richmond[1] has pointed out that a determination of the aldehyde figure for cream will at once show whether a low fat percentage is due to the addition of milk or water.

The aldehyde figure for cream devoid of fat is practically identical with that of skim milk, so that the addition of milk to cream has no effect on the aldehyde figure calculated on the cream devoid of fat, whilst the addition of water naturally at once lowers it.

The aldehyde figure is determined in the usual way (Vol. VIII, p. 154) and calculated to c.c. of $N/1$ strontium or sodium hydroxide per 1,000 c.c. of cream. This figure is then calculated to cream devoid of fat thus:

$$\text{Aldehyde figure} \times \frac{100}{100 - \text{fat}}$$

The average figure for cream is 20.8 c.c., using $N/10$ strontium hydroxide for the titration. If $N/10$ sodium hydroxide be used the figure becomes 15.8° c.c. If the figure obtained is distinctly below 20.8 c.c. (or 15.8 c.c. for sodium hydroxide) the addition of water is indicated.

The above considerations also bear out the contention of Richmond that the solids not fat of cream are in the same ratio to the water present as they are in milk.

In determining the aldehyde figure, Richmond has suggested the use of a standard pink colour for the detection of end points. The standard tint is made by adding to 10 c.c. of milk 1 drop of 0.01% solution of rosaniline acetate in 96% alcohol.

It follows that the acidities determined in this manner will be slightly higher (about 1.5°) than those obtained in the usual manner by titrating till the first pink tinge is observed to be permanent, but the titration for the aldehyde figure, which is a difference only, is not affected and is probably rendered more accurate as the sensitiveness of the observer's eye to a pink colour varies in different individuals.

Gerber Method (Vol. VIII, p. 187).—In order to simplify the introduction of the cream into an ordinary Gerber tube, Bracher uses a small

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[1] *Analyst*, 1914, 39, 243.

glass cup fitted by a solid glass stem into the India-rubber stopper of the tube, similar to those supplied with the special Gerber tube open at each end. The cup is made sufficiently wide to pass into the opening of an ordinary Gerber "bottle" and has a capacity of about 1.25 c.c. This allows about 1 grm. of all creams to be weighed into it. The acid is run into the bottle, followed by 10 c.c. of water and 1 c.c. of amyl alcohol, and then the stopper and cup carefully inserted. The narrow bore of the cup prevents the cream from running out. The test is finished in the usual manner.

Analysis of Dried Milks (Vol. VIII, p. 239).—An extensive investigation into the methods of fat estimation in dried milk has been made by Utz.[1] His conclusions are similar to those arrived at for cheese, and he recommends the Polenske method (see cheese) as the best and quickest. The following analyses are also due to him:

Description	Water	Ash	Fat	Protein. $N \times 6.37$	Sugar. (by diff.)
Whole milk	7.28	5.44	27.72	24.33	35.23
Whole milk	4.14	4.94	37.50	21.40	32.03
Whole milk	7.19	5.65	27.41	25.54	34.21
Whole milk	6.43	5.38	29.71	24.33	34.15
Whole milk	6.33	5.23	26.50	24.97	36.97
Half cream milk	7.31	5.85	20.30	27.65	38.89
Half cream milk	8.00	6.26	14.80	28.99	41.95
Half cream milk	6.69	6.26	14.65	28.54	43.86
Skim milk	9.04	6.96	1.07	30.58	52.35
Skim milk	10.31	7.14	1.92	32.81	47.82
Skim milk	9.05	6.87	0.63	33.25	50.20
Cream	3.65	3.96	51.37	17.65	23.37
Cream	4.17	4.21	43.80	18.98	28.84

These are all probably of German origin.

Analysis of Cheese.

Fat Estimation.—Considerable attention has been given during the last year or two to the analysis of cheese particularly as to its fat content. In order to ensure uniformity of results this question has been fully investigated by several workers, particularly by Utz[2] who considers that the best methods are (1) the Ratzliff-Schmid-Bondzynski and (2) the Polenske.

(1) **The Ratzliff-Schmid-Bondzynski Method.**—3–5 grm. of cheese are placed in a flask with 10 c.c. of hydrochloric acid (sp. gr. 1.125) and boiled gently over a small flame for 8–10 minutes. The solution is cooled and poured into a Gottlieb tube, 25 c.c. of methylated and petroleum ethers respectively added, in that order, mixing the solution gently by inversion after each addition. The ethereal layer is allowed to separate for 6 hours, pipetted off into a weighed flask and the acid liquor extracted once more with the same volume of ethers. The ether in the weighed flask is then distilled off and the fat dried to constant weight.

(2) **The Polenske Method.**—1–1.5 grm. of cheese are placed in a conical flask with 10 c.c. of water and 5 c.c. sulphuric acid (sp. gr. 1.81–1.84) and the mixture heated over a small flame, being allowed to boil gently for

[1] *Milch Zentralbl.*, 1914, 43, 113.
[2] *Milch Zentralbl.*, 1913, 42, 457.

2 minutes, a cooling tube being preferably arranged in the mouth of the flask. 35 c.c. of water are then added, the solution cooled, and 50 c.c. of methylated ether (at 18°) run in and the mixture gently shaken. 50 c.c. of petroleum ether (at 18°) are added and the mixture again gently shaken for 1 minute, and then cooled in water at 18°. After 15-20 minutes 49.5 c.c. of the ether are pipetted off and passed through a cotton wool filter into a small weighed flask. The cotton wool is washed two or three times with a little ether, the ether then distilled off and the fat dried and weighed.

The final proposals (April 27, 1913) of the Committee of the Fédération International de Lâiterie for the unification of analytical methods for cheese are as follows:

(1) **Estimation of Water.**—2-3 grm. of the mixed sample of cheese are quickly weighed into a suitable flat nickel or platinum dish, containing either ignited coarse quartz powder or sea sand purified by hydrochloric acid, and a glass stirrer, and the cheese mixed with the quartz powder or sea sand as equally as possible. The dish is then heated in an oven at 105-110°.

After about 1½ hours the weight is determined and weighings are made at the expiration of succeeding half hours till the weight ceases to diminish. The weight of the cheese residue is taken as that of the *dry substance*, the loss in weight as the water of the cheese.

(2) **Estimation of Fat.**—(A) *By Gerber's method (with modified cheese butyrometer of Van Gulik) for 3 grm. of cheese.*

(A) *Approximate Method.*—3 grm. of cheese are introduced with the aid of a funnel into the widened part of the cheese butyrometer (Fig. 7) which has previously been half filled with sulphuric acid (sp. gr. 1.50). The weighing of very soft cheese is done in a beaker.

The butyrometer is then warmed in a water-bath at 65-70° and the casein dissolved, shaking and swinging the butyrometer at intervals. When no more particles of cheese are visible the butyrometer is left in the water-bath for a short time, swinging at intervals, and then after 1 c.c. of amyl alcohol has been added, it is filled with sulphuric acid nearly up to the mark 35. The contents are mixed with care, not swinging (or shaking) the butyrometer more than necessary to ensure complete mixing, then left for about 5 minutes in the water-bath and whirled at a speed of 1,000 rotations per minute. The diameter of the disc-plate of the Gerber centrifugal machine must be at least 47 cm. (the machine should be warmed when the temperature of the room is low). Finally the butyrometer is placed in a water-bath for 5 minutes at 65° and the height of the fatty layer read at 65°.

(B) *Hydrochloric Acid Method.*—*Final Method.*—1-5 grm.[1] of rasped cheese are gently boiled with 20 c.c. of hydrochloric acid (sp. gr. 1.13) in a small flask, shaking until all small particles have disappeared.

[1] In applying this method it is advisable to use, within these limits, a smaller quantity of fat cheese and a larger one of skim milk cheese.

Then either: (1) *Smetham's percolation method* or (2) the *modified Schmid-Bondzynski method* is applied.

(1) *Smetham's Percolation Method.*—The hydrochloric acid solution, when cool, is carefully introduced into the flask of a Smetham's extractor (Fig. 18), as modified for the estimation of fat in milk, and the flask filled with ether until it flows over.

After extraction for 2 hours the ether is evaporated and the fat dried for half an hour in the oven at 102°. Drying is continued to constant weight.
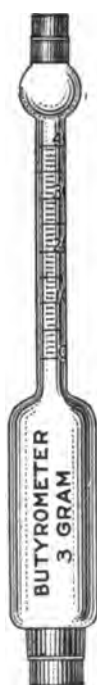
FIG. 17.—Butyrometer, Gerber-Van Gulik. (½ actual size.)

FIG. 18.—Smetham's flask. (⅛ actual size.)

For control a second flask can be connected to the extractor and treated as above.

(2) *Modified Schmid-Bondzynski Method.*—The hydrochloric acid solution is carefully introduced into a 100 c.c. cylinder and, when cool, ether is added until the total volume is about 50 c.c. The cylinder is closed with a damped cork, well shaken, and left for at least 2 hours for the two liquids to separate completely. The ether-fat solution is then drawn off as completely as possible (to at least 1 c.c.) into a weighed flask. A fresh quantity

of 50 c.c. ether is introduced into the cylinder and the contents swung several times. After standing 1 hour the ether is again drawn off as completely as possible and added to the first ether-fat solution in the weighed flask.

The ether is then distilled off and the fat dried for half an hour in the oven at 102°.

Drying is continued to constant weight.

ERRATUM IN VOL. VIII.

Page. 188. The percentage of added water in cream is better given by the following formula:

$$\text{Per cent. added water} = \frac{100f + 950 - 110t}{0.95t - f}$$

ALBUMINOIDS.

By JEROME ALEXANDER.

W. Bachman[1] has investigated the ultramicroscopic structure of jellies with the slit and cardioid ultramicroscopes.

In the case of 1–2% solutions of gelatin, optically homogeneous except for dust particles and similar impurities, the course of gelatinisation exhibited six different phases: (1) a homogeneous light-cone, linearly polarised; (2) a scintillating motion, which gradually resolved into (3) a dense mass of submicrons with a translatory motion; (4) diminution of the Brownian movement of the optical impurities of the gelatin solution; (5) diminution of the translatory motion of the gelatin submicrons and oscillatory motion about a mean position of equilibrium; (6) a differentiated gel with quiescent "jelly elements" (i.e., the smallest structural elements detectable by ultramicroscopic means), which because of their strong linear polarisation of light, are probably aggregations of submicrons and amicrons. The mass of the gel is resolved into ultramicroscopic and microscopic elements, which, in the order of their magnitude, lie near the limit of the power of resolvability of the microscope; these elements may be microscopic in one dimension and ultramicroscopic in another.

Increasing concentration renders the ultramicroscopic heterogeneity of the jellies less and less distinct; jellies of 7–10% concentration appear homogeneous, but the strong polarisation of the diffuse light cone indicates that they are actually heterogeneous and probably composed of extremely fine particles formed into larger jelly elements. The more concentrated the jelly, the closer together these "jelly elements" would be, and the smaller would be their difference in composition and therefore in refractive power between the two phases, so that at a certain concentration the two phases would be indistinguishable even with the ultramicroscope, i.e., the jelly would appear homogeneous. In the case of gelatin solutions of such small concentration that they do not gelatinise (0.5%) flocks are gradually formed owing to the aggregation of isolated "jelly elements" or of submicrons.

The observations in all cases indicate a very much finer structure for jellies than that described by Bütschli. Jellies treated with alcohol or chromic acid showed under the microscope the honeycomb structure of Bütschli, but the structure revealed by the ultramicroscope was a granular ("globulitic") one; and it was found that the alcohol (or chromic acid)

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[1] Zeit. anorg. Chem., 1911, 73, 125–172.

caused coagulation and shrinking (dehydration) and that these are the
cause of the honeycomb structure of Bütschli, the true structure of the
jelly being much finer.[1]

For the *detection of glue* in sizing materials E. Schmidt (*Farber Ztg.*,
24, 97–98) uses ammonium molybdate and a modified Nessler's solution,
which he finds more sensitive than the biuret test or precipitation with
tannin. The special Nessler solution is prepared by making ordinary Nessler
solution slightly acid with sulphuric acid, and filtering to remove the red
precipitate. The clear yellow filtrate is a sensitive test for glue even in the
presence of ammonium salts.

Both of these reagents give a faint turbidity with a solution containing
0.00001 grm. glue in 5 c.c. water. Norgine and albumin interfere with the
test, and must be removed by adding dilute nitric acid to the solution, freed
from fat, and then filtering.

Detection of Gelatin.—The provisional method adopted by the U. S.
Dept. of Agriculture[2] for the detection of gelatin consists in shaking 10
c.c. of the cream with 10 c.c. of acid mercuric nitrate solution, filtering
after 5 minutes, and adding an equal volume of picric acid solution; a pre-
cipitate is produced if gelatin is present. G. E. Patrick[3] pointed out that
sour cream containing no gelatin frequently gives the above reaction, be-
cause of the presence of "pseudo-gelatin" decomposition products. A.
Seidenberg[4] found that the two precipitates may be differentiated by the
fact that while both precipitates are soluble on heating in slightly acid
solutions, only the "gelatin picrate" is soluble in hot neutral water alone.
The picric acid precipitate from sour cream appears to be entirely insoluble
in hot water, after all the picric or other acid has been removed. To ac-
complish this, the picric acid precipitate is shaken vigorously to cause it
to coalesce, and after standing, most of the liquid can be siphoned off. The
precipitate is washed on a filter with water containing 2 or 3 drops of ammo-
nium hydroxide to 100 c.c., until the washings are slightly alkaline to litmus;
it is then boiled with 10–20 c.c. of distilled water and filtered hot into a test-
tube. If gelatin is present, the cooled filtrate will give a decided precipitate
with an equal volume of picric acid solution. By this method it is possible
to detect 0.5% of gelatin in 20–25 c.c. of sour cream.

To separate gelatin from an aqueous solution containing proteins,
albumoses, peptones, mucin and casein, Berrár[5] adds to the solution about
2 volumes of a mixture of 1 part of a saturated aqueous solution of picric
acid with 4 parts of 96% alcohol. The gelatin alone remains in solution,
and after filtration may be quantitatively precipitated by adding about 1½
volumes of saturated aqueous picric acid solution and allowing the mixture
to stand for 12 hours at 10°. By this means he accurately estimated

[1] See also R. Zsigmondy, *Zeit. anorg. Chem.*, 1911, **71**, 356–377.
[2] *Bull.* 107, rev., 121.
[3] *U. S. Dept. Agr.*, *Bull.* 116, p. 24.
[4] *J. Ind. Eng. Chem.*, 1913, **5**, 927.
[5] *Biochem. Zeits.*, 1912, **47**, 189–214.

gelatin mixed with milk and egg albumin. The gelatin picric-acid precipitate was washed with a solution of potassium mercuric iodide until the washings were no longer yellow in colour; the quantity of gelatin in the residue was found by estimating the nitrogen content.

For the qualitative detection of gelatin in the presence of proteins, the filtrate from the alcohol-aqueous picric-acid solution, is tested with picric acid solution, a yellowish-white opalescence being formed even at a concentration of 1 in 100,000. The opalescence is best detected by a "ring-test."

Berrár also found that the solubility of purified gelatin in cold water is 0.56, 0.60 and 0.62% at temperatures of 17°, 18° and 19° respectively. These concentrations approximate to the minimum required for the formation of a jelly; thus a 0.7% solution in hot water sets to a thin jelly on cooling. If a saturated solution, formed in the cold, remains in contact with pieces of gelatin, a partial separation of the dissolved substance occurs upon further cooling, but no gelatinisation. Neither alcohol nor metaphosphoric acid precipitates gelatin completely from aqueous solutions. The same is true of picric acid at ordinary temperature, but at 8° complete precipitation occurs on adding an equal volume of saturated aqueous picric acid solution. When gelatin is completely precipitated by acid reagents, e.g., by a solution containing picric acid and potassium mercuric iodide (the precipitate may in this case be safely washed with water) the gelatin and acid combine in definite proportions, 1 molecular equivalent of acid combining with a quantity of gelatin containing 10 atoms of nitrogen. On the assumption that 1 molecular equivalent of acid unites with 1 molecule of gelatin, the molecular weight of the latter must be 823 (Paal found 900).

The nitrogen content of picric acid or of precipitates containing this compound and proteins or gelatin, may be estimated accurately by a modification of the Kjeldahl method. The material is treated in a Kjeldahl flask, with 0.5–1 grm. of iron filings, 5 c.c. of glacial acetic acid, 20 c.c. of concentrated sulphuric and some copper sulphate; the mixture is heated in the ordinary way, and the subsequent operations carried out as usual.

To replace hide powder in tannin analysis, E. Giusiana[1] proposes the use of fish glue or gelatin previously tanned in a basic chrome solution prepared from 100 grm. of chrome alum crystals, 1,000 c.c. of water and 15 grm. of crystallised sodium carbonate. After soaking for 24 hours in this solution, thin sheets of gelatin are completely tanned and are quite insoluble in hot water. They are washed, neutralised in 1% ammonia, again thoroughly washed and dried between filter papers. The tanned gelatin may be kept indefinitely in distilled water. For 100 c.c. tannin solution the equivalent of 5 grm. of dry gelatin is used. The shakings, filtrations, etc., are the same as in the hide powder process.

[1] *J. Am. Leather Chem. Assoc.*, 1913, **8**, 143.

MEAT AND MEAT PRODUCTS.

By JOHN PHILLIPS STREET.

MUSCLE EXTRACTIVES.

Glycogen.—Trowbridge and Francis[1] obtained the following percentages of glycogen in beef liver and lean beef muscle:

Description of animal	Glycogen in liver	Glycogen in lean muscle
	%	′ %
	Slaughtered 2–3 hours after regular A.M. feed	
Very fat Short-horn, 4½ years...............	3.803	0.663
Lean Jersey cow, 6 years....................	2.337	0.697
Fairly fat Short-horn, 3½ years.	1.210	0.243
Fat yearling Hereford	0.927	0.375
Fat Hereford, 20 months...................	0.820	0.309
	Slaughtered 5–6 hours after regular A.M. feed	
Thin Hereford, 2 years......................	0.965	0.569
Very thin Hereford, 23 months..............	0.913	0.416
Thin Hereford, 11 months..................	0.625	0.158
	Slaughtered 9 hours after regular A.M. feed	
Very fat Hereford, 3 years..................	1.160	......
Fairly fat Hereford, 18 months..............	0.284	0.140

The above data show the greater tendency of older animals to store glycogen and the influence of the length of time elapsing after feeding before the animal is slaughtered on the amount of glycogen that remains stored in the organs and muscles. Furthermore, the glycogen-content of the muscle of the emaciated Hereford steer (0.416%) shows that notwithstanding this emaciated condition, and the fact that the entire carcass contained no fat which could be separated mechanically, the muscle still contained glycogen in considerable quantity.

Trowbridge and Francis also obtained (*loc. cit.*) the following percentages of glycogen in cow liver and horse flesh at various periods of time after slaughter.

Liver of cow	Glycogen	Horse flesh	Glycogen
	%		%
After 2 hours 13 minutes	3.13	After 36 minutes......	0.146
After 1 week...........	2.67	After 22 hours........	0.072
After 15 days..........	2.34	After 3 days..........	0.013

These experiments show that the glycogen-content of beef muscle and beef liver ranges from 0.1 to 0.7, and from 0.2 to 3.8%, respectively; that starvation or extreme debility does not cause complete removal of glycogen from the muscle or liver; that the glycogen slowly decreases, but does not entirely disappear when the meat is kept at a temperature of 6.5° C. for over

[1] *J. Ind. Eng. Chem.*, 1910, **2**, 215.

two weeks; that glycogen may be present even when the liver is unfit for food; that horse flesh is subject to an enzymatic hydrolysis of the glycogen similar to that of beef; and finally that the glycogen content cannot be said to offer an absolute or even approximate basis for distinguishing beef from horse flesh.

Estimation of Glycogen.—Starkenstein[1] calls attention to a source of error in Pflüger's method. Ferric hydroxide, which may be present, is capable of absorbing large quantities of glycogen. The insoluble portion obtained after treating the tissue with potassium hydroxide should be dissolved in hydrochloric acid and the glycogen estimated in the solution. Unless this is done the error may amount to as much as 50% of the total glycogen present.

Bierry and Gruzewska[2] suggest the following method, which they claim gives excellent results:

Place 25 grm. of finely hashed meat in a flask containing 25 c.c. of potassium hydroxide solution. Heat for 15 minutes till the tissue is completely dissolved, then in an autoclave at 100° for 30 minutes. Cool, neutralise to litmus with hydrochloric acid, make up to 100 c.c., and heat in an autoclave at 120° for 30 minutes. Cool and neutralise with sodium hydroxide. Precipitate the proteins with mercuric nitrate. Filter the liquid brought up to 300 c.c. with the wash water and add zinc powder to remove excess of mercury. After several hours a colourless liquid is obtained. Estimate the dextrose in a 10 c.c. aliquot and multiply by 0.927 to obtain the weight of glycogen.

Estimation of Lactic Acid.—Meissner[3] concentrates the lactate solution to a syrup, then treats with 5 c.c. of phosphoric acid and rubs with dry calcium sulphate to form a powder. This is extracted with ether for 7–9 hours, thus removing the lactic acid quantitatively. The extract is concentrated and the acid titrated, or estimated as carbon monoxide by conversion into the barium salt, concentrating to dryness and decomposing with sulphuric acid in an atmosphere of carbon dioxide. The decomposition is usually complete at 100°, but the flask may be heated to the boiling point of sulphuric acid.

Yoshikawa[4] estimates *d*-lactic acid by measuring the rotation of its lithium salt.

Detection of Inositol.—All of the inositols upon oxidation with nitric acid yield coloured oxyquinone derivatives. In carrying out this test Scherer's method[5] is generally used. Treat a small amount of the material to be tested with a little nitric acid and evaporate on a water-bath almost to dryness; add a little ammoniacal barium chloride or calcium chloride, and again evaporate the solution. If inositol is present, a beautiful rose-red colour will develop; 0.5 mg. of inositol may be detected.

[1] *Biochem. Zeit.*, 1910, 27, 53.
[2] *Compt. rend.*, 1913, 156, 1491.
[3] *Biochem. Zeit.*, 1915, 68, 175.
[4] *Zeit. physiol. Chem.*, 1913, 87, 382.
[5] *Liebig's Ann.*, 1850, 73, 322; 1852, 81, 375.

Seidl[1] has modified the test by using ammoniacal strontium acetate to develop the colour and in this way 0.3 mg. may be detected.

Salkowski[2] has still further modified the Scherer test as follows: Dissolve a small amount of the substance in 1-2 drops of nitric acid, add a few drops of 10% calcium chloride solution, then a few drops of 1-2% platinic chloride solution, and evaporate the whole in a porcelain dish. The presence of inositol is indicated by a rose-red to brick-red colour, the test being sensitive to 0.1 mg.

Nitrogenous Muscle Extractives.

Occurrence.—Wilson[3] has reviewed in detail the work of recent years on the nitrogenous extractives of muscle. The following is a summary of the various nitrogenous compounds thus far found in the muscular tissue of both vertebrate and invertebrate animals:

Amino-acids.—*Alanine.*—In beef extracts and the muscle of lobster, salmon, tunny, snapper, crab and dried codfish.

Glycocoll.—In scallop, mussel, crab extract and shell fish; none in octopus.

Glutamic Acid.—In beef extract and dried codfish.

Leucine.—In crustacea, cephalopods, spiders and insects; specifically in crab extract, lobster, cuttlefish, sardines and crab.

Tyrosine.—In crab extract, lobster, sardines, snapper, crab and many invertebrates.

Histidine.—In beef extract, bonito, tunny, salmon and sardines.

Arginine.—In crab extract, lobster, crab and clam.

Lysine.—In crab extract and lobster.

Proline.—In lobster.

Tryptophane.—In crab.

Taurine.—In oyster, cuttlefish, octopus, frog, alligator, cephalopods, mollusca, periwinkle, abalone, beef extract and dried codfish.

Dipeptides.—*Anhydride of* d-*alanyl*-d-*alanine.* In beef extract.

Carnosine (Ignotine).—In beef extract, muscles of ox, bonito, tunny, salmon, eel, calf, rabbit, sardine, horse and pig. None found in liver or kidney extracts. Several investigators have failed to obtain it from invertebrate muscle.

Methylguanidine.—In beef extract and muscle of ox, haddock, calf, horse and codfish. It has been found in liver extract but not in kidney extract..

Creatine and Creatinine.—In the muscle of practically all vertebrates.

Trimethylamine.—In fish and invertebrate extracts. It is possibly present only in small amounts in living muscle.

Choline.—In beef extract, crab extract, dogfish and in many plant and animal tissues.

[1] *Chem. Zeit.*, 1887, 11, 676.
[2] *Zeit. physiol. Chem.*, 1910, 69, 478.
[3] *Chemistry of the Nitrogenous Extractives of Muscle Tissues.* Thesis, Yale Univ., 1914.

Neurine.—In beef extract and haddock.

Carnomuscarine.—In beef extract.

Betaïne.—In sugar beet and many plant and animal extracts; also in muscle extracts of shrimp, crab, oyster, clam, dogfish, cuttlefish, octopus, mussel, dried codfish, scallop, periwinkle and lamprey.

Neosine.—In beef extract, crab extract and ox muscle.

Carnitine.—In beef extract and muscle of ox, calf, horse and pig; not in kidney or liver extracts.

Myokynine.—In dog and horse.

Oblitine.—In beef extract.

Purines.—*Hypoxanthine, xanthine, guanine and uric acid.* In muscle extracts.

Carnine.—In beef extract and horse muscle.

Inosinic acid.—In beef extract.

Miscellaneous Compounds.—*Methylamine.* In flesh of coot.

Iminazolethylamine.—In tunny.

Urea.—Probably present in small amount in all muscle extracts. Unusually large amounts in muscle extracts of cartilaginous fishes.

Compounds of Unknown Structure.—*Vitiatine and Creatosine.*—In beef extract.

Canirine.—In crabs and snapper.

Crangitine and Crangonine.—In crab extract.

Melolonthine.—In cockchafer.

Wilson gives in his thesis an extensive bibliography of muscle extractives. The reader is also referred to the papers by Ackermann, Becker, Blaha, Bottazzi, Buglia and Constantino, Cabella, Demjanowski, Dietrich, Einbeck, v. Fürth, Gulewitsch, Jansen, Jona, Krimberg and Israilsky, Mauthner, Mendel, Micko, Myers and Fine, Smorodinzew, Suzuki, Wilson and Yoshimura, which have appeared during the last three years, chiefly in *Zeit. physiol. Chem., J. Biol. Chem., Biochem. Zeit.,* and *Amer. J. Physiol.*

Estimation of Nitrogenous Extractives.

Creatine and Creatinine.—The Folin method with its various modifications continues to be the standard method for determining these meat bases. Wieland[1] has pointed out that the salts of acetic acid, semi-carbazide, dioxyguanidine and quinol give a red colour with picric acid and soda. Sudendorf and Lahrmann[2] have made a similar observation, and have modified the method by using a 1% solution of potassium permanganate, which removes the interfering substances. After this treatment tomato juice, yeast extract, caramel and acetone did not give the Jaffé reaction.

Thompson, Wallace and Clotworthy[3] recommend creatinine picrate or

[1] *Konserven-Zeit.,* 14, 249.
[2] *Zeit. Nahr. Genussm.,* 1915, 29, 1.
[3] *Biochem. Jour.,* 1913, 7, 445.

the double picrate of creatinine and potassium for control of the standard dichromate solution in the Folin method. In estimating creatinine in weak solutions, the best results were obtained by using an equal quantity of $N/1$ hydrochloric acid, and heating either on the water-bath for 3 hours or in the autoclave for 25 minutes at 117° C. (identical results were obtained by both methods of heating). The optimum time and temperature for the development of the colour were 7 minutes and 15–17° C. For accurate work the readings on the colour scale are strictly proportional only if they lie between limits of 7 and 9 mm. Dextrose to the extent of 10% does not affect the estimation of creatinine.[1]

Folin himself has discarded potassium dichromate as the colour standard, preferring creatinine zinc chloride. His own most recent modification of the method of estimating creatine in muscle is as follows: Transfer 5 grm. of comminuted muscle to a 200˙c.c. Erlenmeyer flask and add 100 c.c. of $N/2$ sulphuric acid. Cover the flask with tin foil and heat in the autoclave at 130–135° for 30–40 minutes. After cooling to below 100° open the autoclave, cool the contents of the flask and transfer to a 200 c.c. flask. Shake for a short time to break up the skeletal tissues, dilute to 200 c.c. and mix well. Filter and titrate 10 c.c. of the filtrate with 10% sodium hydroxide using phenolphthaleïn as indicator. To another 10 c.c. portion in a 100 c.c. flask add 20 c.c. of saturated picric acid and enough sodium hydroxide solution to give 1.5 c.c. in excess of that required to neutralise the sulphuric acid. As standards use a solution containing creatinine equivalent to 1 mg. of creatine per c.c. (1.389 grm. creatinine zinc chloride) for striated muscle, and standards half as strong for other muscle. In the former case set the standard at 10 mm., in the latter at 20 mm. In either case 4,000 divided by the reading of the unknown in millimeters gives the creatine in milligrams per 100 grm. of muscle.[2]   (See also p. 565.)

Phosphorus Compounds.

Forbes and Keith[3] have published a very extensive (709 pages) review of the literature of phosphorus compounds in animal metabolism, to which the reader is referred. A very complete bibliography is given.

Inorganic Phosphorus.—In estimating inorganic phosphorus in meat and eggs, Chapin and Powick[4] extract the inorganic phosphoric acid by using approximately $N/10$ hydrochloric acid with an excess of picric acid, the estimation being made in aliquots of the filtrate. In case the amount of water in the sample is not known and the volume of the material insoluble in the extracting liquid cannot be neglected, a known amount of potassium iodide may be added as a "marker," and from the concentration of potassium iodide in the extract the degree of dilution by the water in the sample

[1] Cf. also Baur and Trümpler, Zeit. Nahr. Genussm., 1914, 27, 697.
[2] See J. Biol. Chem., 1914, 17, 463–493.
[3] Ohio Agr. Expt. Stat., Tech. Bull. No. 5, 1914.
[4] J. Biol. Chem., 1915, 20, 97.

may be calculated. The potassium iodide is estimated by Schirmer's nitrous acid—urea method.[1] The phosphoric acid is first precipitated with magnesia mixture and then as ammonium phosphomolybdate by Lorentz's method. As eggs deteriorate, as judged by physical means, there is a progressive increase in the ratio of inorganic to total phosphorus.

Tin in Canned Foods.

Baker[2] obtains tin sulphide in the usual way from 100 grm. of canned food (digesting with nitric and sulphuric acids and precipitating with hydrogen sulphide). This is then dissolved in hydrochloric acid with the addition of potassium chlorate; a few pieces of aluminum foil are then added to the boiling solution to eliminate all the chlorine, and the tin is reduced to the metallic state by adding about 1 grm. of aluminum foil, this and subsequent operations being carried out while an atmosphere of carbon dioxide is maintained over the surface of the liquid. The mixture is again heated, the tin dissolving to form stannous chloride and, after the addition of air-free water, the solution is titrated with $N/100$ iodine solution, using starch as indicator.

Decomposition of Meat.

Ottolenghi[3] has reviewed the methods proposed to trace the ripening of meat and to ascertain when active decomposition begins. Only Sörensen's method[4] of estimating amino-acids and the microscopical examination for bacteria were found to be of practical use, though Chodat's tyrosinase-p-cresol reaction and the lowering of the freezing point and the condition of the serum gave reliable results but were too time-consuming and required a too elaborate manipulation. The amount of amino-acids (expressed as N) should not exceed 350 mg. per 100 grm. of fat- and tendon-free meat dried at 70°. Well-kept meat on this basis usually contains 190–320 mg., and meat in incipient state of decomposition 340–700 mg. As marked decomposition approaches, ammonia is formed, which interferes with the estimation, causing low results unless first removed by distillation.

Estimation of Ammonia.

Folin's method[5] for the determination of ammonia in urine by means of an air current is applicable to the estimation of ammonia in meat foods. The most recent modification of this method[6] is as follows (compare p. 564):

Arrange five vessels in series as follows: (1) A bottle containing sulphuric acid, with a Hopkins safety bulb, to purify the entering air; (2) a litre flask containing 25 grm. of sample, 250 c.c. of water, 5 grm. of sodium chloride and 1 grm. of sodium carbonate (alcohol may be added to prevent foaming);

[1] Arch. Pharm., 1912, 250, 448.
[2] 8th Inst. Congr. Appl. Chem., 1912, 18, 35.
[3] Zeit. Nahr. Genussm., 1913, 26, 728.
[4] Biochem. Zeit., 1908, 7, 43, 407.
[5] Zeit. physiol. Chem., 1902–03, 37, 161.
[6] U. S. Dept. Agr., Bur. of Chem., 1913, Circ. 108, 10.

(3) a 250 c.c. safety flask; (4) a cylinder, fitted with a Folin absorption tube, containing $N/10$ sulphuric acid; (5) a 100 c.c. safety flask. The last flask should be connected with an air pump powerful enough to draw the ammonia over into the standard acid. Alcohol may be substituted almost wholly for the water if the air current is weak. Titrate the standard acid at intervals of an hour until no more ammonia is given off, carrying out a control experiment at the same time. Methyl-red, cochineal or congo red may be used in aqueous solution, methyl-red or cochineal in alcoholic.

Frozen Meat.

Wright[1] has studied the changes in New Zealand lamb and mutton when exposed to temperatures from 2° to 19° F. for periods up to 160 days. A progressive increase was noted in the soluble nitrogen, this increasing from 21.7 to 75.5% after 14 days. The ammoniacal nitrogen rose from 0.8 to 53% of the total nitrogen. Under cold storage a loss of from 2.5 to 3.5% of moisture was observed, with an increase of proteose, peptone and meat bases, and a decrease in coagulable nitrogen, ammoniacal nitrogen remaining unchanged.

Meat Extracts.

In the list of papers given on page 396, Vol. VIII, no reference is made to the extensive investigation of commercial meat extracts made by Street[2] A very complete bibliography of the subject is given in the above report. The following are Street's maximum and minimum values for thirty-five brands of paste and fluid preparations:

	Paste preparations		Fluid preparations	
	Max.	Min.	Max.	Min.
Water	36.54	14.79	68.37	42.03
Organic matter	77.90	51.56	41.07	19.05
Ash	36.28	14.45	21.56	11.28
Petroleum ether extract	0.50	0.04	0.62	0.00
Chlorine	17.81	2.50	11.44	2.99
= added salt[3]	25.05	0.00	15.84	2.02
Phosphoric acid	6.22	1.15	2.87	0.88
Potash	12.65	2.29	4.81	1.73
Acidity,[4] phenolphthalein	14.50	3.74	6.64	2.40
Acidity,[4] litmus	9.07	1.90	4.24	1.18
Nitrogen, total	10.47	5.02	5.36	1.78
Nitrogen insoluble	0.34	0.00	0.99	0.00
Nitrogen, coagulable	0.26	0.00	0.10	0.00
Nitrogen, ammonia	0.74	0.13	0.58	0.11
Nitrogen, ppt. by tannin salt	7.89	2.93	3.20	0.64
Nitrogen, meat bases	4.37	0.63	2.30	0.80
Nitrogen, ppt. by zinc sulphate	5.04	0.44	1.59	0.31
Nitrogen, creatinine	1.85	0.07	0.49	0.00
Nitrogen, creatine	1.30	0.03	0.48	0.90
Nitrogen purine	0.83	0.16	0.36	0.07
Nitrogen, undetermined meat bases	1.87	0.14	1.76	0.36

[1] *J. Soc. Chem. Ind.*, 1912, 31, 965.
[2] *Conn. Agr. Expt. Stat. Rept.*, 1908, 606-672.
[3] See Allen's *Comm. Org. Anal.*, 1913, Vol. VIII, 394.
[4] c.c. $N/10$ KOH per gram of sample.

ANALYSES OF PROPRIETARY MEAT PREPARATIONS.

Brand	Water	Alcohol by weight	Organic matter	Ash	Petroleum ether extract	Chlorine	Phosphoric acid	Potash	Acidity c.c. N/10 KOH per gram of material		Forms of nitrogen							Meat bases			
									Phenol-phthalein	Litmus	Total	Insoluble	Coagulable	Ammonia	Ppt. by tannin-salt	Meat bases	Ppt. by zinc sulphate	Crea-tinine	Crea-tine	Purines	Undeter-mined
Bovinine	68.21	6.33	30.18	1.61	0.09	0.74	tr.	0.18	0.38	0.34	2.57	0.06	0.14	0.04	2.26	0.07	2.39	0.00	0.00	0.03	0.04
Mason's essence of beef	88.18	0.00	10.46	1.36	0.09	0.14	0.42	0.62	0.98	0.35	1.30	0.00	0.05	0.06	0.85	0.34	0.77	0.08	0.05	0.05	0.16
Valentine's meat juice	55.72	0.00	32.75	11.53	0.10	0.90	2.67	5.18	6.92	4.34	3.13	0.05	0.00	0.26	0.90	1.92	0.12	0.22	0.13	0.13	0.20
Wyeth's beef juice	53.81	0.00	29.02	17.17	0.02	4.75	3.06	4.48	6.60	4.72	3.25	0.12	0.41	0.19	0.85	1.68	0.20	0.18	0.11	0.16	1.23
Liquid peptonoids	68.43	13.05	30.66	0.91	0.06	0.22	0.12	0.18	0.76	0.30	0.83	0.00	0.00	0.05	0.58	0.20	0.27	0.02	0.03	0.00	0.15
Pepto-mangan "Gude"	81.17	13.25	17.98	0.85	0.01	tr.	tr.	0.02	0.26	0.17	0.16	0.00	0.02	0.12	0.07	tr.	0.04	0.00	tr.	0.00	0.00
Colden's liquid beef No. 2	68.85	16.53	30.98	0.17	0.04	0.04	tr.	0.09	0.20	0.11	0.05	0.00	0.00	0.04				0.03	0.02	0.03	0.24
Panopepton	61.82	14.19	37.04	1.14	0.00	0.32	0.18	0.33	2.02	1.08	1.12	0.05	0.00	0.04	0.72	0.36	0.29	0.06	0.06	0.03	0.24
Mulford's predigested beef	74.30	12.52	25.32	1.38	0.00	0.26	0.02	0.10	1.44	0.80	0.50	0.00	0.00	0.00	0.31	0.19	0.13	0.02	0.03	0.03	0.12
Murdock's liquid food	73.53	8.60	25.75	0.72	0.30	0.19	0.04	0.14	1.10	0.80	1.98	0.39	0.00	0.00	1.18	0.28	1.28	0.16	0.00	0.00	0.28
Asparox	68.37	0.00	17.12	14.51	0.02	6.10	0.90	2.27	2.40	1.64	1.78	0.10	0.07	0.11	0.64	0.92	0.38	0.13	0.16	0.00	0.49
Vigoral	43.15	0.00	41.14	15.71	0.19	3.54	2.85	5.34	6.64	3.84	4.09	0.26	0.00	0.16	1.53	1.97	0.55	0.46	0.15	0.31	1.05
Bovox	51.74	0.00	30.25	18.01	0.29	8.53	0.88	1.76	3.04	1.18	4.02	0.06	0.00	0.16	2.80	1.00	1.58	0.19	0.10	0.07	0.64
Bovril	43.12	0.00	41.07	15.81	0.62	4.78	2.48	3.55	2.97	3.10	5.36	0.99	0.04	0.45	2.33	1.55	1.33	0.49	0.30	0.30	0.41
Cibil's fluid extract	64.21	0.00	19.95	15.84	0.00	7.11	1.38	1.98	4.32	2.00	2.79	0.14	0.00	0.18	1.40	1.07	0.60	0.28	0.35	0.17	0.44
Maggi's bouillon	49.34	0.00	29.32	21.34	0.00	1.44	1.01	1.64	4.68	2.28	2.88	0.00	0.00	0.18	0.82	1.88	0.33	0.05	0.01	0.06	1.76
Sinuox	57.33	0.00	26.76	15.91	0.00	7.10	1.36	2.37	4.32	3.28	2.80	0.00	0.00	0.58	0.83	0.87	0.57	0.29	0.04	0.18	0.36
Sinuox for invalids	51.57	0.00	29.51	18.92	0.16	7.55	2.18	3.48	5.92	3.56	2.70	0.00	0.05	0.28	0.95	1.42	0.31	0.42	0.22	0.26	0.52
Somatose	11.19	0.00	82.72	6.09	0.09	0.02	1.47	0.23	1.70	1.50	12.64		0.00	0.14	12.19	0.26	10.60	0.00	tr.	0.04	0.22
Mosquera beef meal	12.58	0.00	82.23	5.19	8.56	0.60	1.45	2.24	6.10	2.30	12.25	6.73	0.00	0.26	4.15	1.11	0.63	0.17	0.11	0.04	0.79

Street also reports (*loc. cit.*) the analyses of certain proprietary meat preparations as shown on preceeding page.

Micko[1] has continued his extensive studies on meat extracts and bouillon cubes, and in the papers referred to discusses exhaustively the methods of analysis and the basis of interpretation of the results secured.

Einbeck[2] has isolated from meat extract. succinic acid and fumaric acid,[3] but could not establish the presence of malic acid. Krimberg and Izrailsky[4] isolated creatinine.

Salkowski[5] points out that zinc chloride and sodium carbonate precipitate purine bases, but from flesh extracts colloids which interfere with further isolation of the bases may also be precipitated. The colloids may be destroyed by heating the extract with dilute nitric acid before the zinc chloride treatment.

Smorodinzew[6] compared the yield of meat bases obtained by various subsulphate methods and found that treatment with a 10% mercuric solution in 5% sulphuric acid and precipitation of the filtrate, after removal of mercury, with phosphotungstic acid gave the best yields of purine bases and carnosine. The addition of sulphuric acid considerably reduced the yield of carnosine; the addition of lead salts likewise reduced the yield of carnosine and only slightly improved that of methylguanidine. Purine bases are not completely precipitated by phosphotungstic acid and their precipitation is apparently unaffected by lead salts or sulphuric acid.

Smorodinzew found ox flesh to contain 0.024% of purine, 0.265 of carnosine, 0.051 of methylguanidine and 0.029% of carnitine. He also found that extract of mutton contained twice the amount of purines and nearly twice the amount of carnitine found in beef extract, but only one-third the carnosine and one-half the methylguanidine. Mutton also contains more purines and carnitine and less carnosine and methylguanidine than horse flesh.

Partition of Nitrogen in Meat Extracts.

Cook[7] has continued his studies on means of differentiating plant, yeast and meat extracts, using the methods previously employed together with the following method of Rippetoe for nitrogen precipitated by acid aclohol:

Nitrogen Precipitated by Acid-Alcohol.—Transfer 10 c.c. of an aqueous solution of the extract (= 1 grm.) to a 200 c.c. glass-stoppered measuring cylinder, add 1.2 c.c. of 12% hydrochloric acid, mix, add absolute alcohol to the mark, mix thoroughly and set aside for several hours at 20–25°. If necessary, make up to mark, filter and transfer 100 c.c. to a Kjeldahl flask, evaporate the alcohol on a water-bath and determine the nitrogen in the residue.

[1] *Zeit. Nahr. Genussm.*, 1913, 26, 321; 1914, 28, 489.
[2] *Zeit. physiol. Chem.*, 1913, 87, 145.
[3] Same journal, 1914, 90, 301.
[4] Same journal, 1913, 88, 324.
[5] *Biochem. Zeit.*, 1913, 55, 254.
[6] *Zeit. physiol. Chem.*, 1914, 92, 214, 221.
[7] *J. Amer. Chem. Soc.*, 1914, 36, 1551.

Cook found no creatine or creatinine and very little purine nitrogen in the plant extracts. The yeast extracts showed high purines and no creatine or creatinine. Plant and yeast extracts did not give the biuret reaction. All of the nitrogen of the plant extract was found in the filtrate after applying acid-alcohol, in the form chiefly of mono-amino acids and ammonia. About 25% of the nitrogen of the meat and yeast extracts is precipitated by acid-alcohol. The plant extracts yield a much larger percentage of ammoniacal nitrogen than the meat or yeast extracts.

PARTITION OF NITROGEN OF PLANT, YEAST, AND MEAT EXTRACTS.

					Percentage of total nitrogen				
	Total nitrogen	Ammonia nitrogen	Total creatinine nitrogen	Purine nitrogen	Nitrogen in phosphotungstic acid filtrate	Nitrogen in acidalcohol filtrate	Nitrogen in tannin salt filtrate	Amino-nitrogen	
								Formol method	Van[1] Slyke method
Meat extracts.	9.56	2.62	22.49	3.35	7.64	78.04	54.91	10.94	18.50
	9.65	2.49	22.59	3.52	6.84	78.45	55.85	10.94	18.23
	7.68	1.56	32.42	2.86	71.35	100.00	73.04	10.63	15.63
	9.65	....	29.01	4.92	49.74	76.73	59.37	10.27[2]	15.54
	7.49	....	27.50	0.13	....	89.19	64.21	9.53	17.89
Yeast extract.	5.35	2.43	0	11.22	49.96	72.15	56.07	17.64	32.71
Plant extracts.	5.23	7.46	0	0.42	85.85	100.00	94.46	36.71	65.77
	6.34	12.78	0	0.44	84.08	99.69	89.95	36.43	57.41
	6.33	....	0	0.25	....	100.00	83.41	30.64	45.18
	6.27	11.32	0	0.44	83.25	98.56	92.66	35.88	67.78
	6.47	6.03	0	....	63.68	100.00	84.54	30.75	44.20
	6.08	12.98	0	0.46	84.04	99.67	92.76	35.85	61.18
	6.63	10.56	0	0.23	86.88	99.25	91.40	34.08	57.31

Bouillon Cubes.

Many analyses of these preparations have been reported and methods worked out for estimating the percentage of meat extract present.[3]
Cook[4] gives the following analyses of certain typical brands:

ANALYSES OF BOUILLON CUBES.

Brand	Solids, per cent	Organic matter, per cent.	Ether extract, per cent.	Ash, per cent.	Total chlorine as NaCl, per cent.	Total phosphoric acid, per cent.	Acidity (c.c. N/20 KOH per grm.)	Total nitrogen, per cent.	Nitrogen ppt. by acidalcohol, per cent.	Total creatinine, per cent.
Behrend...	96.60	22.86	1.93	73.74	72.13	1.02	6.20	2.19	0.13	0.84
Oxo.......	95.06	25.31	3.10	69.75	65.00	1.51	6.50	2.97	0.86	1.07
Steero.....	96.05	28.41	1.20	67.64	62.15	1.83	9.15	3.62	0.76	1.67
Burnham..	96.87	41.94	1.00	54.93	52.90	0.58	6.10	2.11	0.05	0.88
Sunbeam..	95.73	45.23	1.44	50.50	49.26	0.54	7.30	2.36	0.02	0.92
Armour....	96.05	26.48	0.96	69.57	67.44	0.62	6.00	2.79	0.17	1.07
Morris.....	96.77	33.00	3.79	63.77	59.17	1.69	9.68	3.67	0.56	1.07
Standard..	95.81	21.76	4.19	74.05	72.22	0.48	5.01	2.09	0.07	0.90
Liggitt.....	96.00	21.91	4.58	74.09	71.98	0.41	4.75	2.11	0.05	0.49
Knorr.....	95.44	26.24	4.57	69.20	65.00	1.55	7.40	3.20	0.91	1.38

[1] Not corrected for 15% of the ammonia nitrogen.
[2] Not corrected for ammonia nitrogen.
[3] See Cook, *J. Amer. Chem. Soc.*, 1914, 36, 1551, and Micko, *Zeit. Nahr. Genussm.* 1913, 26, 321; 1914, 27, 489.
[4] *J. Ind. Eng. Chem.*, 1913, 5, 989.

According to Serger,[1] if we place the creatinine content of meat extract at 6%, then bouillon cubes should contain the following amounts of creatinine for the respective amounts of meat extract used:

Meat extract used, per cent.	Creatinine contained, per cent.
25	1.5
20	1.2
15	0.9
10	0.6

Geret[2] found from results based on the analysis of 100 samples that the meat extract of bouillon cubes varied from 20 to 25% in the best cubes to none at all in the inferior grades, calculating the solids of high-grade meat extract at 80%. The cubes contain about 70% of water, fat and sodium chloride and 30% of any one or any combination of meat extract, plant extract and plant seasoning compound, the last named consisting of amino-acids derived from proteins by heating with acid until no biuret reaction is given, and then neutralising with soda.

Serger[3] claims that bouillon cubes should contain not more than 8% of water, 9 of fat, 65 of sodium chloride, and not less than 15% of meat extract. Good cubes contain between 92 and 98% of solids, 3.0 to 3.9 nitrogen, 0 to 5 sugar, 0.9 to 1.5 creatinine, 62 to 67 ash, 1.2 to 1.7 phosphoric acid, 18 to 25 protein and a ratio of N/P of 2.3 to 2.5.

Remy[4] analysing 10 high-grade samples found 12.3 to 17.8% of mineral matter (of which 11.3 to 16.2 was sodium chloride), 10.7 to 17 water and 2.1 to 10.6 fat. Inferior products containing as high as 60% of sodium chloride have been observed.

Kapeller and Gottfried[5] found 9 samples to range from 3.4 to 7.2% water, 4.4 to 10.9 fat, 9.8 to 24.8 albuminoids, 54.6 to 76.6 ash and 51.6 to 74.4 sodium chloride. The same chemists analysed 8 other samples as follows:[6]

Water	Albuminoids	Fat	Ash	Sodium chloride
6.5	13.4	6.7	65.9	63.3
3.3	14.6	8.2	56.3	54.9
4.4	14.1	6.2	69.1	66.2
3.4	10.1	5.7	77.2	74.5
3.8	9.6	8.3	70.4	69.4
3.3	6.6	10.5	73.1	72.5
5.6	6.0	7.0	70.6	69.1
1.1	0.5	2.9	83.7	83.0

Conti[7] analysed 3 samples as follows, the last consisting largely of gelatin and salt:

[1] *Konserven-Zeit.*, 48, 378.
[2] *Zeit. Nahr. Genussm.*, 1912, 24, 570.
[3] *Zeit. öffent. Chem.*, 1914, 20, 80, 101.
[4] *Pharm. Zentralh.*, 1913, 54, 1238.
[5] *Zeit. Nahr. Genussm.*, 1913, 26, 161.
[6] *Zeit. Nahr. Genussm.*, 1914, 28, 224.
[7] *Boll. chim. farm.*, 1913, 51, 183.

Water	Organic matter	Nitrogen	Fat	Ash	Sodium chloride	Phosphoric acid
6.5	17.0	2.48	4.2	71.9	65.0	1.06
7.0	22.0	2.49	7.1	63.0	60.0	0.40
22.0	51.5	7.64	....	26.5	24.0	0.58

Krasser[1] found that Maggi's bouillon cubes during the years 1908–1911 ranged from 52.4 to 56.6% water, 21.9 to 27.2 organic matter, 20.0 to 25.7 albuminoids, 20.4 to 21.6 ash, 17.0 to 18.7 sodium chloride and 0.87 to 1.13 phosphoric acid.

Street[2] gives the following analyses of bouillon cubes and other condensed soups:

	Water	Fat	Ash	Protein	Carbo-hydrates	Nitrogen	Sodium chloride
Knorr's pea soup with bacon.....	9.6	9.0	15.0	21.4	45.0	3.42	12.5
Knorr's bean soup..............	11.0	6.2	16.0	19.3	47.5	3.09	12.8
Knorr's consommé..............	4.3	5.4	66.5	....	....	3.10	61.5
Steero bouillon cubes...........	6.6	1.7	65.2	....	....	3.89	59.5
Oxo bouillon cubes.............	4.8	3.6	67.5	....	....	3.28	62.7
Armour's beef extract and vegetable tablets.................	10.2	0.4	29.6	....	....	1.63	22.4
Mason's beef tea lozenges.......	10.8	0.1	9.7	....	....	[3]13.04	3.3
Anker's bouillon capsules........	8.6	0.1	47.1	....	....	5.54	38.4

Soups.

Street[4] analysed 6 brands of canned bouillon showing the following range of composition (over 99% of 1 brand consisting of water and salt):

	Max.	Min.	Ave.
Water..	96.4	92.0	94.6
Ash..	2.7	1.1	1.9
Ether extract.................................	0.15	0.06	0.09
Protein (N × 6.25)............................	2.09	0.49	1.39
Sodium chloride...............................	2.48	0.93	1.63
Nitrogen.....................................	0.334	0.079	0.222
Undetermined.................................	4.21	0.33	2.05

Congdon[5] has also investigated certain condensed soups. The amount of meat fibre in chicken soup ranged from 2.18 to 16.48%, the liquid portion from 6.01 to 83.88 and the other ingredients (chiefly boiled rice) from 13.94 to 90.32%. He showed that the average composition of 8 brands of "condensed" chicken soup was as follows:

Boiled rice...................................... 27.18
Meat extract.................................... 3.54
Meat fibre..................................... 8.10
Salt... 2.05
Water... 59.13

[1] Zeit. Nahr. Genussm., 1914, 27, 78.
[2] Conn. Agr. Expt. Stat. Rept., 1908, 660; 1911, 161; 1914, 238.
[3] Contained much gelatin.
[4] Conn. Agr. Expt. Stat. Rept., 1910, 493.
[5] No. Dak. Food Dept. Spec. Bull., 1913, 2 (15), 246; 1914, 3 (5), 62

On the original basis these samples ranged from 81.4 to 94.1% water, 0.94 to 4.13 protein (N × 6.25), 0.07 to 1.96 water-soluble protein, 0.06 to 1.33 fat, 1.17 to 12.03 carbohydrates, 1.09 to 3.12 ash, and 0.92 to 2.83 salt. In some instances more beef fibre was present than chicken.

Sausage.

Water-content.—Feder,[1] after analysing a large number of authentic samples, declares that the water should not exceed 60%. The ratio of water to fat-free organic matter is the most reliable index for added water, and should never exceed 4. This judgment has been confirmed by Schenck,[2] who found samples containing as much as 80% of water.

Estimation of Benzoic Acid.—Krüger[3] maintains that most of the previously proposed methods give unsatisfactory results in substances containing a large proportion of protein, difficulty being experienced in extracting the whole of the acid from such foods. He suggests the following method:

Mix 50 grm. of the finely divided meat with 45 c.c. of 70% sulphuric acid and submit to steam distillation. Collect 500 c.c. of the distillate, the flask being heated so as to maintain the volume of the contents as constant as possible. Filter the distillate, make slightly alkaline with sodium hydroxide and evaporate to a small volume. Heat the residual liquid on a water-bath and add potassium permanganate drop by drop until the pink colour remains for 5 minutes. Destroy the excess of permanganate with sodium sulphite, evaporate the mixture to 10 c.c., transfer to a separating funnel and acidify with sulphuric acid, rinsing the evaporating dish with sodium sulphite solution and dilute sulphuric acid and adding the washings to the funnel. Extract the acid solution, which should not exceed 20 c.c., with ether; wash the ethereal extract with water, allow the solvent to evaporate spontaneously in a weighed dish and weigh the residue after drying for 2 hours over soda-lime. The dried residue may be dissolved in alcohol and titrated with $N/10$ sodium hydroxide. If the weight of benzoic acid is less than 30 mg., Polenske's sublimation method[4] should be used as a control.

Composition of Meat Rations.

Gephart and Lusk[5] have made a valuable study of 242 ready-to-serve foods as dispensed by a well-known chain of restaurants in New York City. The various food portions were analysed, their calorific values determined, and a summary given of the cost of 2,500 calories in the various kinds of food purchased.

[1] Chem. Zeit., 1914. 38, 709; Zeit. Nahr. Genussm., 1913. 25, 577.
[2] Zeit. Nahr. Genussm., 1915, 29, 145.
[3] Zeit. Nahr. Genussm., 1913, 26, 12.
[4] Analyst, 1911, 36, 584.
[5] Analysis and Cost of Ready-to-serve Foods, Chicago, 1915.

Eggs.

Colouring Matter of Yolk.—Barbieri[1] has shown this to be ovochromin, which decomposes at 270° and is a yellow, hygroscopic powder, soluble in its own weight of water, but insoluble in ordinary organic solvents. It is decolourised by hydrogen peroxide, but alkalis and concentrated acids have no action on it in the cold.

Sugar Content.—Morner[2] found that the sugar content, mainly glucose, of egg white varies from 0.3 to 0.5% in the common egg. In eggs of other species the lowest sugar was 0.12, the highest 0.32, and the average of 51 varieties 0.22%.[3]

Estimation of Lecithin.—Cohn[4] suggests the following method:

1 to 2 grm. of commercial lecithin preparations or 5 to 20 grm. of food containing lecithin are extracted for several hours with two successive por- .tions of 100 c.c. of 96% alcohol, the first extraction at ordinary temperature, the second at the boiling temperature of alcohol, a reflux condenser being used. The residue is ground with sand, extracted once more with alcohol, and then boiled for 2 hours with about 100 c.c. of chloroform. When dealing with fatty substances it is advantageous to extract with chloroform immediately after the cold alcohol extraction. In certain cases the extraction with hot alcohol must be continued for 20 hours in order to extract all the phosphorus compounds. Evaporate the alcohol and chloroform extract, boil the residue for 2 hours with 100 c.c. of chloroform to separate the lecithin from glyceryl-phosphoric acid and free phosphoric acid; filter the solution and evaporate. The amount of phosphorus in the residue is estimated by oxidising with nitric and sulphuric acids, or igniting it with the addition of magnesium oxide, or a mixture of sodium carbonate and potassium nitrate, precipitating the resulting phosphoric acid with molybdic acid solution and continuing in the usual way.

Estimation of Salicylic Acid in Preserved Eggs.—Froidevaux[5] has pointed out that salicylic acid cannot be determined in preserved eggs by the usual procedure. If a mineral acid is used to liberate salicylic acid, an unfilterable magma results. If the magma be treated with ether to extract the salicylic acid, emulsions form, and fats, lipochromes, lecithin, etc., pass into the solvent. He proceeds as follows:

To 25 grm. of powder or 30 grm. of liquid egg contained in a 500 c.c. porcelain dish 250 c.c. of water are added, the mixture stirred, 125 c.c. of 8% sodium hydroxide solution added and the mixture warmed for 45 minutes on the water-bath. The resultant gelatinous mass is broken up with a glass rod and the particles washed with water by decantation and on the filter. The filtrate is acidified with hydrochloric acid and 20 c.c. of sodium phospho-

[1] *Compt. rend.*, 1912, **154**, 1726.
[2] *Zeit. physiol. Chem.*, 1912, **80**, 430.
[3] See also Bierry, Hazard and Ranc, *Compt. rend. soc. biol.*, 1914, **73**, 93.
[4] *Zeit. öffent. Chem.*, 1913, **19**, 54.
[5] *J. pharm. chim.*, 1915, **10**, 18.

molybdate solution added to precipitate the protein. The filtrate from this is extracted with ether in the usual way. The method is sensitive to 0.0023 grm. in 100 grm. of material.

Distinguishing between the Whites of Hen and Duck Eggs.—Waterman[1] prepared sera by injecting the white of hen eggs and duck eggs respectively into rabbits and drawing off the blood a week after the last injection. These sera were standardised by adding to 0.1 c.c. of the serum 1 c.c. of standard solutions of white of hen and of duck eggs, respectively, ranging in strength from $\frac{1}{1.000}$ to $\frac{1}{80.000}$. A precipitate is formed at the junction of the liquids. The greatest dilution at which this precipitate is formed is taken as the titre of the serum. The whites of the two kinds of eggs should react only with their respective sera. The sample to be tested is made up to various concentrations and treated as above. When the sample is a mixture it will react with both sera. One analysis showed 60% of white of duck eggs and 40% of hen egg white.

Estimation of Albumin.—Labbé and Maguiso[2] have proposed a volumetric method in which the egg albumin is precipitated by the citropicric acid reagent prepared according to Esbach.

Fish.

Composition.—Williams[3] gives very complete analyses of 25 varieties of English fish.

Anchovy Butter.—Behre and Frerichs[4] claim that true anchovy butter is characterised by a lower fat content and lower iodine number and refraction of the extracted fat than herring butter, or a mixture of the two. In general, anchovy butter containing less than 10% of fat contains no foreign fish; that containing about 15% of fat must be regarded as suspicious if the iodine number exceeds 60 and the refraction 50. The addition of foreign fish cannot be detected in mixtures containing equal parts of fish and butter.

Nitrogen of Fish Muscle.—Wilson[5] has studied the partition of the nitrogen in extracts of the muscles of the lamprey, limulus, squid, clam, scallop and periwinkle. Betaïne was isolated from the scallop, periwinkle and lamprey; creatine from the lamprey.

Okuda[6] obtained the following results in grams per 100 grm. of dry substance:

[1] *Chem. Weekblad.*, 1913, 11, 120.
[2] *Compt. rend.*, 1913, 156, 1415.
[3] *Chem. News*, 1911, 104, 271.
[4] *Zeit. Nahr. Genussm.*, 1912, 24, 676.
[5] *Jour. Biol. Chem.*, 1914, 17, 385; 1914, 18, 17.
[6] *8th Int. Cong. App. Chem.*, 1912, 18, 275.

	Creatine	Creatinine
Bonito...	2.01	0.48
Tunny fish.......................................	1.80	0.23
Salmon..	1.53	0.18
Snapper...	3.33	0.31
Carp..	2.02	0.37
Shark...	3.24	0.66
Lobster, crab, clam and cuttlefish................	trace	trace

Yoshimura and Kanai[1] found dried codfish to yield per kilogram 1.4 grm. of creatinine, 0.44 betaïne hydrochloride, 0.70 methylguanidine picrate, 13 taurine, 0.50 alanine, traces of glutamic acid, and no creatine or choline.

Glycogen in Fish.—Schöndorff and Wachholder[2] found the glycogen content of fish muscle to range from none to 0.68%. '

Caviare.—König and Groszfeld[3] found fish roe to contain xanthine, hypoxanthine, creatinine, taurine, *l*-tyrosine, glycocoll, thymine, proteins soluble and insoluble in water, and fat. The proteins are rich in sulphur and phosphorus and do not yield protamines. The fat is characterised by a high lecithin content (up to 59%) and also contains from 3.9 to 14% of cholesterol.[4]

Helen Chernoruzkii[5] found 1.2 grm. of nucleic acid in 100 grm. of freshly dried and alcohol-ether extracted herring eggs.

Kodama[6] has shown that by means of the precipitin, anaphylaxis (active and passive) and complement-binding reactions, caviare can be differentiated from other fish spawn, such as carp, red eye, bream, tench, salmon, herring and trout. By means of the precipitin reaction the fish-roe protein can be clearly differentiated from the fish protein of the same animal.

Dinslage[7] found a sample of caviare preserved with urotropin (hexamethylentetramine), which he identified by means of Rimini's method as modified by Arnold and Mentzel.[8]

[1] *Zeit. physiol. Chem.*, 1913, **88,** 346.
[2] Pflüger's *Arch. Physiol.*, 1914, **157,** 147.
[3] *Biochem. Zeit.* 1913, **54,** 338, 351.
[4] For detailed analyses see above paper and also *Zeit. Nahr. Genussm.*, 1914, **27,** 502.
[5] *Zeit. physiol. Chem.*, 1912, **80,** 194.
[6] *Arch. Hyg.*, 1913, **78,** 247.
[7] *Zeit. Nahr. Genussm.*, 1913, **26,** 200.
[8] *Zeit. Nahr. Genussm.*, 1902, **5,** 353.

FIBROIDS.

By W. P. DREAPER, F. I. C.

ESTIMATION OF SMALL QUANTITIES OF WOOL IN COTTON MATERIALS.

This is a matter of some importance under modern conditions. The wool is separated by P. Heerman[1] in the following manner: Sulphuric acid (80%) dissolves cotton completely in 2–3 hours, whilst wool only loses 1.5% of its weight in the same time. In practice the sample (5–10 grm.) is thoroughly extracted with ether and then with 96% alcohol, and the fibre is then treated with ten to twenty times its weight of the acid. After standing 6 hours the cotton has completely dissolved. The liquid is diluted with water (cold) and any wool present remains undissolved; it may be dried at 105° after a thorough washing with water and weighed (an allowance of 17% being made for moisture).

[1] *Chem. Zeit.*, 1913, 1257.
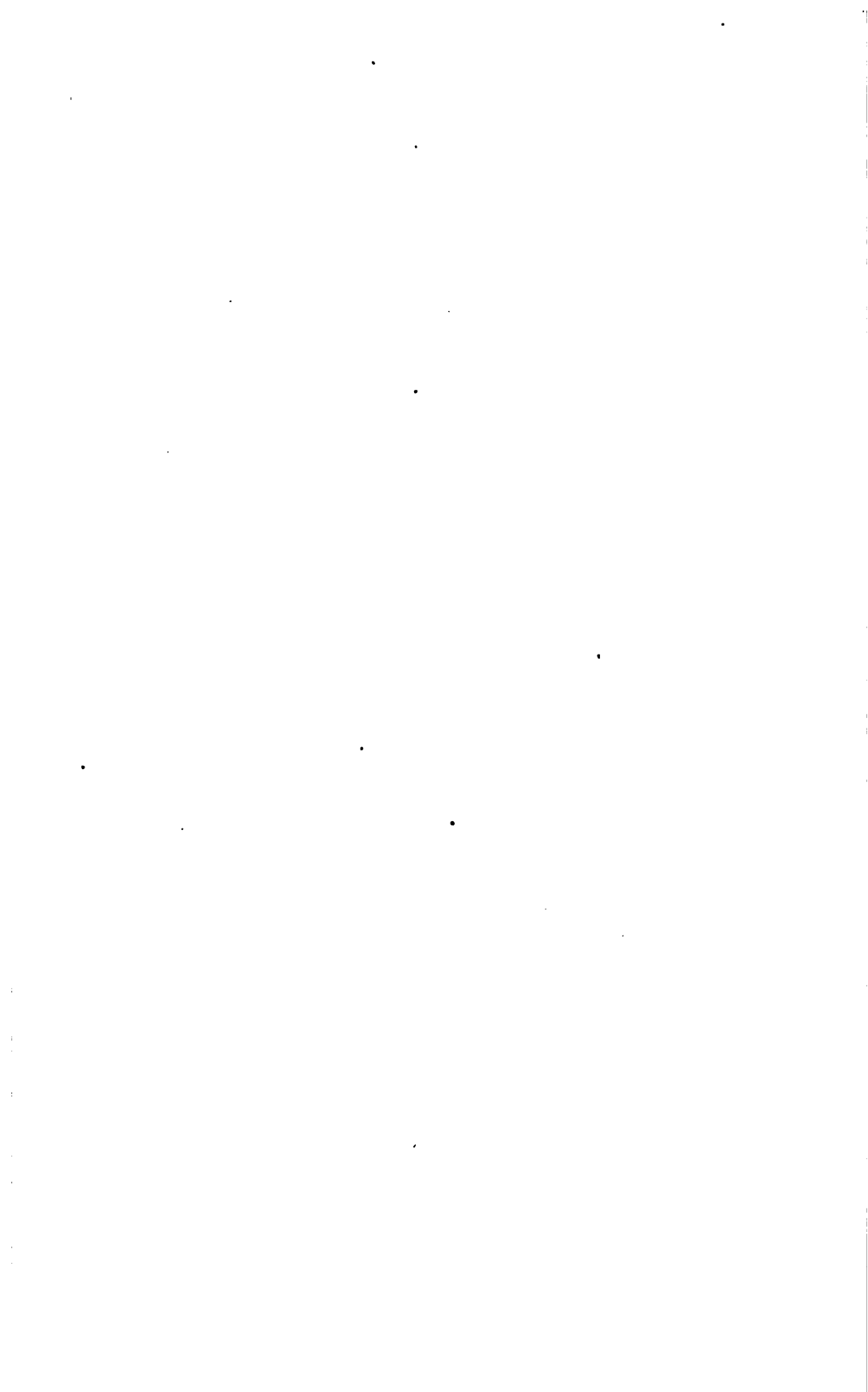
APPENDIX

Sugars.—For the use of enzymes and special yeasts in carbohydrate analysis reference should be made to a paper by W. A. Davis in the *J. Soc. Chem. Ind.*, 1916, 35, 201. This deals with the question of the estimation of saccharose, raffinose, maltose and starch. For estimating raffinose, the material proposed by Hudson & Harding (*J. Amer. Chem. Soc.*, 1915, 37, 2193), using autolysed top and bottom yeasts, is at the present moment probably the most accurate and most convenient. Regarding the supposed precipitation of reducing sugars by basic lead acetate see Davis (*J. Agric. Science*, 1916, 7, 255).

For the estimation of small quantities of reducing sugars in presence of large quantities of saccharose see Maquenne (*Compt. rend.*, 1915, 161, 617; 1916, 162, 145, 207 and 277). For details of the estimation of sugars in plant material and a discussion of the question of the formation and translocation of sugars in the tissues see Davis (*J. Agric. Sci.*, 1916, 255, 328, 352); for the question of the presence of maltose in leaves and in germinated barley and its influence on the measurement of amylolytic power, see Davis (*Biochemical J.*, 1916, 31).   W. A. D.

Butter.—It has been the experience of several observers, besides the writers, that + values for the "difference figure" have been obtained in the case of several butters of undoubted purity during the later months of last year (1915). These butters are particularly of Irish origin and the probable reason is the difficulty of obtaining proper feeding stuffs arising out of war conditions. Butters from sources not so affected have not shown any departure from the normal.   C. Revis.

Arginine.—Clemanti (*Atti. R. Accad. Lincei*, 1914, 23, ii, 517 and 611) has shown that arginase, an enzyme present in liver extracts, converts arginine quantitatively into urea and ornithine. It is probable that this reaction will afford a convenient means of estimating arginine, either following the change by measuring the new amino-group produced by a formalin titration method (Clementi) or by determining the urea produced by the urease method (Plimmer). For a discussion of the ordinary method of estimating arginine see Plimmer (*Biochemical J.*, 1916).   W. A. D.

Meat Extracts.—For the methods adopted in the estimation of nitrogenous constituents of extracts derived from albuminous substances such as meat extracts with special reference to the interpretation of the results see the report of the discussion on this subject in the *Analyst*, July, 1915.

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